Acinetobacter septicus sp. nov. Association with a Nosocomial Outbreak of Bacteremia in a Neonatal Intensive Care Unit

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Acinetobacter species other than Acinetobacter baumannii have rarely been reported to be associated with nosocomial outbreaks of bloodstream infections. Within a period of 1 week, seven Acinetobacter-like isolates were recovered from peripheral blood and catheter specimens of five patients at a neonatal intensive care unit (NICU) in a tertiary hospital in Turkey. All five patients had placement of central venous catheters and had received total parenteral nutrition before the onset of bacteremia. Two of the five patients died. Medical devices, tap water, aerators, water samples, various surfaces, intravenous fluids, and the hands of health care workers in the NICU were sampled and were culture negative for the bacterium. All seven of the isolates had identical biochemical reactions, antimicrobial susceptibility results, and pulsed-field gel electrophoresis patterns, indicating a clonal nosocomial outbreak. A panel of standard biochemical reaction profiles and three phenotypic commercial identification systems failed to identify these isolates. Phenotypically, the isolate differed from Acinetobacter ursingii by its hemolysis on sheep blood agar and its negative citrate utilization. Sequences of the full 16S rRNA gene, which contained at least three different gene copies with polymorphic sequences between nucleotide positions 70 and 206, were determined from the first recovered isolate. The complete 1,529- to 1,531-bp 16S rRNA gene sequences and partial 801-bp rpoB gene sequences had similarities of 99.5% and 97.2%, respectively, to an A. ursingii isolate. The DNA-DNA similarities of the strain against the type strain of A. ursingii were 64.7 and 68.7%, which were lower than the recommended threshold value of 70% for the definition of bacterial species. These data indicate that a novel Acinetobacter organism caused the nosocomial outbreak of bacteremia in the NICU unit. We propose the designation of Acinetobacter septicus sp. nov. for these isolates, with isolate AK001 as the type strain.

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with a nosocomial outbreak of bacteraemia in a neonatal intensive care unit (NICU). Within a period of 1 week, seven Acinetobacter-like isolates were recovered from peripheral blood and catheter specimens of five patients at a NICU in Gulhane Military Medical Academy Hospital in Turkey. All seven isolates had identical biochemical reactions, antimicrobial susceptibility results, and pulsed-field gel electrophoresis (PFGE) patterns, indicating a clonal nosocomial outbreak. Identification systems based on biochemical reactions failed to identify these isolates. Sequence analysis of full 16S rRNA and partial rpoB genes and DNA-DNA hybridization suggest that these isolates are new Acinetobacter species, and the designation of Acinetobacter septicus sp. nov. is proposed.

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MATERIALS AND METHODS

Patients and participants. Gulhane Military Medical Academy Hospital is a 1,500-bed teaching hospital in Ankara, Turkey. Three doctors, four nurses, and one nurse trainee were in the 15-bed NICU. Patients with low birth weight or respiratory dysfunction related to prematurity are generally admitted to this NICU. During 1 week in March 2006, a total of seven Acinetobacter isolates were recovered from five of eight newborn patients’ blood specimens. Informed consent was obtained from the parents of all five patients. Clinical specimens were collected, and medical records were reviewed. Samples from medical devices, tap water, aerators, water samples, various surfaces, intravenous fluids, and the hands of health care workers were collected and cultured to evaluate potential risk factors and to find a source of the bacteraemia.

Specimen processing and bacterial isolation. Patients’ blood samples were processed with the Bectec 9240 nonradiometric blood culture system (Bectec Dickinson, Sparks, MD). When the positive blood cultures were confirmed to be gram-negative bacilli by Gram stain, they were subcultured onto MacConkey and other media. Susceptibility testing were performed using Trimod TraceIdentPro version (version 1.1; Ridom GmbH, Würzburg, Germany). Multiple alignment, sequence similarity of the 16S rRNA and partial rpoB genes, and neighbor-joining trees with bootstrap values were calculated using MEGA 3.1 software (27). Both full 16S rRNA and partial rpoB gene sequence trees were group rooted with Pseudomonas aeruginosa strain PAO1 sequences.

DNA-DNA hybridization. DNA-DNA hybridization of strain AK001 against A. seuticur (DMS 16307) was performed at the DSMZ. Bacterial DNA was isolated from logarithmic-phase cultures (Thermo Spectronic, Madison, WI) and was purified by chromatography on hydroxyapatite as described previously (4). DNA-DNA hybridization was carried out in 2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) at 67°C (8), according to modifications described previously (18), using a Cary 100 Bio UV/VIS spectrophotometer equipped with a Peltier-stabilized 6-by-6 multichannel and a temperature controller with an in situ temperature probe (Varian Inc., Palo Alto, CA). A threshold value of 70% DNA-DNA similarity was used for the definition of bacterial species according to ad hoc committee recommendations (44).

Nucleotide sequence accession numbers. The sequences of the full 16S rRNA (three operons) and partial rpoB genes of A. seuticur AK001 (DMS 19415) were deposited in the GenBank database under accession numbers EF611418 to EF611429 and EF611383.

RESULTS

There were eight patients in the NICU during the study period. A total of seven unusual Acinetobacter isolates, from five blood and two catheter tip samples, were recovered from a total of five newborns. All seven isolates had the same antibiotic susceptibility patterns and were susceptible to the antibiotic that was used in this study, except for cefazidime and cefotaxime. Two of the five neonates died; the others recovered after a week’s course of intravenous amikacin and meropenem (Table 1). The same Acinetobacter strain was not isolated from environmental and personnel samples, which were sampled at the same time period.

All seven strains had the same biochemical test results, including hemolysis on sheep blood agar, nonfermentative, nonmotile, catalase positive, oxidase negative, citrate-negative, urase negative, and indole and H2S production negative. A full panel of standard biochemical reactions were performed on the first isolate, AK001, which was a Gram stain-negative rod with a width and length of 0.7 to 0.9 and 1.0 to 2.5 μm, respectively. It produced hemolysis on sheep blood agar and was lysed by 3% KOH. It was positive for aminopeptidase and alcohol dehydrogenase but negative for conversion from NO3 to NO2. Other key biochemical reactions are listed in Table 2.

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This unusual *Acinetobacter* isolate was differentiated from *A. ursingii* by its hemolysis on sheep blood agar and negative citrate reaction (34, 36). Biochemical reactions were the same for all seven isolates by use of the bioMerieux API 20E, Remel Rapid NF Plus, and Biolog GN2 systems: all three systems failed to make an identification with acceptable similarity scores. The epidemiologic relatedness of the seven isolates was further analyzed by PFGE. For comparison, one *A. baumannii* strain, which was isolated from the burn unit at Vanderbilt University Hospital multiple times, was included (7). The PFGE patterns of the seven isolates were identical, suggesting that the isolates were epidemiologically related and clonal in origin (Fig. 1).

The 16S rRNA genes for all seven isolates were amplified by PCR, and the direct sequencing on amplicons was performed. Mixed sequences around nucleotide position 100 were repeatedly produced. Therefore, amplicons of strain AK001 were subcloned, and a total of 12 clones were selected and subjected to DNA sequencing. Sequencing resulted in three different operons of the 16S rRNA gene, named TAAK0106, TAAK0110, and TAAK0112, respectively, ranging from 1,529 to 1,531 nucleotides. The three full 16S rRNA gene sequences had 99.98% similarity among them and were closely related to an *A. ursingii* isolate (DSM 16037) (34, 36) at 99.5% similarity (Fig. 2). To further characterize the seven unknown *Acinetobacter* strains, partial 801-bp *rpoB* direct gene sequencing was performed.
applied, and the sequences were compared to the sequences of all 17 type strains. All seven unknown Acinetobacter strains showed identical rpoB sequences. Therefore, only AK001 was included in Fig. 3. The sequence of AK001 showed similarities of 97.2% to A. ursingii (DSM 16037) and 76.6 to 81.2% to other Acinetobacter species (Fig. 3).

DNA-DNA hybridization of strain AK001 against A. ursingii (DSM 16037) was performed in duplicate, and the resulting DNA-DNA similarities were 64.7 and 68.7%, which were both lower than the recommended threshold value of 70% for the definition of bacterial species (44). Based on all physiological and chemotaxonomical data, we propose the designation of Acinetobacter septicus sp. nov. for the novel species in the Acinetobacter genus, with strain AK001 as the type strain.

DISCUSSION

In this report, we have described a cluster of five cases of bacteremia within a week in an NICU that were caused by a novel Acinetobacter species. Seven isolates were recovered from peripheral blood and catheter specimens of these five patients. Two of five patients died; the others recovered after receiving a week’s course of intravenous amikacin and meropenem. All seven isolates presented identical biochemical reactions, antimicrobial susceptibility profiles, and PFGE patterns, indicating a clonal nosocomial outbreak.

Bacteremia among newborns in NICUs causes considerable mortality and morbidity and accounts for approximately 30% of hospital-acquired infections in this population (28). The
The incidence of bacterial infection in NICUs is estimated to be about 1 to 8 newborns per every 1,000 live births and 160 to 300 newborns per 1,000 live births in very-low-birth-weight newborns. Coagulase-negative staphylococci and Enterobacter species are the pathogens most commonly isolated from the NICU (31). Acinetobacter spp. are usually considered to be nonpathogenic to healthy individuals; however, especially in debilitated individuals and patients in intensive care units, they do cause nosocomial infections (13). Premature and low-birth-weight infants as well as the length of hospital stay are significant risks for developing infections (33). In this study, three patients were premature and had low birth weights.

Acinetobacter species are widely distributed in nature and in the hospital environment. It has been shown that the digestive tract of intensive care unit patients is an important epidemiologic reservoir in hospital outbreaks (6). Environmental contamination of various hospital items has often been identified, ranging from suctioning equipment to pillows and mattresses (13). Foreign bodies such as catheters play an important role in the pathogenic occurrence of A. Iwofii bacteremia (40, 43). Hand organism carriage by health care workers has been implicated in outbreaks of Acinetobacter infections (2). During an outbreak of A. baumannii bacteremia in an NICU in Taiwan, multiple A. baumannii isolates were recovered from hand washing samples, and some of them were epidemiologically related to those recovered from patients’ blood, suggesting that the hospital environment was the potential reservoir and that transmission was possibly via the hands of health care workers (17). Higher device-associated infection rates and higher device utilization ratios in an ICU were reported in Turkey (20). In our event, all five patients had placement of central venous catheters and had received total parenteral nutrition before the onset of bacteremia. We suspected that cross-contamination of Acinetobacter via the hands of staff members was the likely source of this outbreak. Immediately after the cluster of bacteremia cases were observed, medical devices, tap water, aerators, water samples, various surfaces, intravenous fluids, and the hands of health care workers in the NICU were sampled and were culture negative for the bacterium. The route of transmission of this Acinetobacter bacteremia outbreak remains unknown.

Identification of Acinetobacter isolates to the species level has been problematic in clinical microbiology services. The majority of genospecies cannot be reliably separated by phe-
nototypic tests (37). Some Acinetobacter species present inert biochemical reactions, which makes accurate identification based on phenotypic profiles difficult. In our study, standard biochemical reactions and three phenotypic identification systems, including the API 20E, RapID NF Plus, and Biolog GN2 systems, were unable to identify unusual Acinetobacter isolates to the species level. 16S rRNA gene sequencing determinations have been widely used to identify gram-negative bacilli, including Acinetobacter species (16, 41). However, this technique may fail to distinguish closely related genomic species of Acinetobacter (19, 29). Several studies have demonstrated the usefulness of rpoB gene sequences for the identification and taxonomic classification of various bacterial species including Acinetobacter (30, 35). Our data, based on both 16S rRNA and rpoB gene amplification and sequencing, indicated that the unusual Acinetobacter species was most closely related to A. ursingii, at similarities of 99.5% and 97.2%, respectively. Considering significant differences in several key biochemical reactions, a standard DNA-DNA hybridization method was used to further characterize and contrast the unusual Acinetobacter isolate from the A. ursingii type strain. The DNA-DNA hybridization of strain AK001 against the type strain of A. ursingii resulted in similarities that were below the recommended value of 70% for the definition of bacterial species (44). It is worthwhile to point out that Acinetobacter species are a group of organisms which include naturally competent species and published genomes that reveal a large amount of mobile genes; DNA-DNA hybridization can be expected to vary significantly within bacterial species of this type.

**Description of A. septicus sp. nov.** A. septicus was named to indicate its clinical relevance as an isolate causing sepsis in humans, especially newborns. The bacteria are gram-negative bacilli with inert biochemical activities. Two key phenotypic characteristics, hemolysis on sheep blood agar and negative citrate utilization, were the only differences between A. septicus and A. ursingii. Commercial biochemical identification systems were not useful to identify the bacterium. Genotypically, A. septicus is most closely related to A. ursingii based on nucleotide sequence analysis of both the 16S rRNA and rpoB genes. DNA-DNA hybridization against A. ursingii gave results below the recommended species delineation threshold. A. septicus has been found only in human blood and is considered to be a pathogen that caused a nosocomial sepsis outbreak in an NICU.

**Description of the type strain.** The type strain of A. septicus is AK001 (DSM 19415). It was isolated from the blood of a newborn boy in Ankara, Turkey.

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**REFERENCES**


