

Nosocomial Bacteremia Due to an As Yet Unclassified *Acinetobacter* Genomic Species 17-Like Strain

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We describe a case of bacteremia due to an as yet unclassified *Acinetobacter* genomic species 17-like strain. The recognition of this microorganism as non-*Acinetobacter baumannii* may have important epidemiological implications, as it relieves the hospital of the implementation of barrier precautions for patients infected or colonized as may be necessary with a multiresistant *A. baumannii* epidemic.

CASE REPORT

A 49-year-old man was admitted to a hospital in Barcelona (Spain) on 29 July 2000 because of L2-L3-L4 vertebral fractures as a consequence of a car accident. The patient was a heavy smoker (60 cigarettes a day) and consumed >20 g of alcohol daily. Several days later, renal insufficiency, ascites, and generalized edema developed. A diagnosis of hepatic insufficiency (probably related to alcoholic liver disease) with hepatorenal syndrome was made. On deterioration of the condition of the patient 1 month later, he was transferred to Hospital Bellvitge, also in Barcelona, where repeated paracenteses were performed and intravenous albumin administered, with some improvement. During hospitalization, the patient received piperacillin-tazobactam for primary bacteremia due to *Pseudomonas aeruginosa* over 15 days and ceftriaxone as empirical treatment for a low-grade fever, which was discontinued after negative blood cultures were obtained. Sixty days after admission, the patient presented an acute picture of fever with chills and hypotension, without focal signs or symptoms. Piperacillin-tazobactam was again started, a peripheral catheter (that had been in place for 8 days) was removed, and hemodialysis was begun due to worsened renal insufficiency. Two consecutive blood cultures were processed with an interval of 2 h. Both blood cultures yielded a gram-negative rod that was identified as *Acinetobacter baumannii* in the local laboratory and *Acinetobacter* genomic species 17 by amplified ribosomal DNA restriction analysis (ARDRA) (5) and 16S rRNA gene sequence analysis. However, the genomic fingerprint obtained by amplified fragment length polymorphism (AFLP) analysis

could not be identified by comparison to those of >200 reference strains (see below). Ceftriaxone was substituted for piperacillin-tazobactam, as the organism was susceptible to the former. The bacteremic episode was resolved. Renal biopsy showed a diagnosis of immunoglobulin A mesangial glomerulonephritis with extracapillary reaction. The patient was discharged 5 months after admission. He died 2 months later as a consequence of liver failure with metabolic encephalopathy and gastrointestinal bleeding.

The genus *Acinetobacter* has a complex taxonomy with up to 32 described named and unnamed genomic species (3, 14, 16). Reliable identification of *Acinetobacter* strains to species level is difficult and requires the use of molecular methods or a combination of genotypic and phenotypic methods (14). The species most frequently isolated from clinical samples are *A. baumannii* and *Acinetobacter* genomic species 3 and 13TU which, together with the environmental species *Acinetobacter calcoaceticus*, are combined in the so-called *A. calcoaceticus*-*A. baumannii* complex. The epidemiology and clinical relevance of *Acinetobacter baumannii* has been extensively studied (1), but there is scarce information about the epidemiology and clinical relevance of other, rarely isolated species. This is partly due to the lack of easy methods for species identification. Here we describe a case of bacteremia due to a strain identified by 16S rRNA gene sequence analysis as *Acinetobacter* genomic species 17-like but which could not be classified to any species by AFLP analysis.

The strain (Ac209, LUH8320) was sent to the reference laboratory (Hospital Clinic, Barcelona, Spain) for possible inclusion in the GEIH Ab-2000 project, a nationwide cohort study of all cases of *A. baumannii* colonization or infections in Spain performed during November 2000. The specific meth-

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odology of the study and some results have been published elsewhere (8, 17). In the Laboratory of Microbiology of the Hospital Clinic in Barcelona, Spain, and in the Department of Infectious Diseases of the Leiden University Medical Center, the strain was identified as an unnamed genomic species 17 using ARDRA. The profile, consisting of the combination of restriction patterns 1, 2, 1, 2, and 3 generated with the respective enzymes CfoI, AluI, MboI, RsaI, and MspI, together with BsmAI pattern 2 and with BfaI pattern 4, identified the organism as *Acinetobacter* genomic species 17 strains. With 16S rRNA gene sequence analysis, the microorganisms had a sequence similarity of 99.09% to *Acinetobacter* genomic species 17. With the high-resolution genomic fingerprinting analysis using AFLP, the isolate did not cluster at or above the 50% level with the reference strains of any described (genomic) species and was thus considered an as yet undescribed species (15). Further phenotypic studies were performed according to the method of Bouvet and Grimont (4) with minor modifications (14). The strain showed hemolytic activity and produced gelatinase, while it did not acidify Hugh-Leifson medium with D-glucose. It grew at 37°C but not at 44°C in brain heart infusion broth. Its carbon source utilization profile was different from those of all of the 32 *Acinetobacter* strains described (5, 6, 8, 14). The most notable feature was its ability to grow on histamine, a feature reported so far only in strains of the nonproteolytic genomic species 10 and 11 (4), proteolytic genomic species 14TU (9), and an as yet unclassified proteolytic strain (5). In addition, it did not utilize DL-lactate and 4-aminobutyrate (5), thereby distinguishing it from genomic species 17.

Antimicrobial susceptibility testing was performed by microdilution following the guidelines established by the CLSI (formerly NCCLS) (13). The antimicrobial agents used were ampicillin, piperacillin, cefoxitin, cephalothin, ceftazidime, cefepime, sulbactam, ampicillin plus sulbactam, imipenem, meropenem, ciprofloxacin, gentamicin, tobramycin, amikacin, doxycycline, and polymyxin B. *Pseudomonas aeruginosa* ATCC 27853 was used as a control strain.

Most *Acinetobacter* bacteremias are caused by *A. baumannii*. However, some cases may be caused by other species. Bacteremia due to *Acinetobacter* genomic species 13TU (12), *Acinetobacter junii* (10), and *Acinetobacter ursingii* (11) (identified by molecular methods) have been described. In the present study, a severe clinical case of an as yet unclassified hemolytic *Acinetobacter* strain is described; this case presented as a primary bacteremia with clinical evidence of sepsis in a predisposed patient. The infection responded to antibiotic therapy and removal of a venous catheter (which was the suspected origin of the bacteremia).

Correct identification of *Acinetobacter* species is relevant for therapeutic reasons, as species other than *A. baumannii* are generally susceptible to more antimicrobial agents. The microorganism of the present study was susceptible to all the antimicrobial agents tested except for cefoxitin and cephalothin, while a slightly elevated MIC of polymyxin B (2 mg/liter) was observed. Moreover, the recognition of this microorganism as non-*A. baumannii* may have important epidemiological implications, as it relieves the hospital from implementation of barrier precautions that might be necessary with a multiresistant *A. baumannii* epidemic.

The organism was identified by the widely used commercial API20NE system as *A. baumannii*, while 16S rRNA gene analysis identified the organism as similar to the unnamed genomic species 17. This emphasizes that correct identification of *Acinetobacter* species, according to the current taxonomy, is problematic. The problems of phenotypic identification of acinetobacters has been documented (2, 9), but the usefulness of 16S rRNA gene sequence analysis has not been evaluated yet. In addition, ARDRA profiles, which are essentially based on the 16S sequence, are not always conclusive, since some profiles may occur in different species (7). AFLP fingerprint analysis, using the Leiden University AFLP library of 200 reference strains of all described species, did not identify the strain as any of the 32 species of *Acinetobacter* described. These problems underscore the problem of correct identification of *Acinetobacter* species in the diagnostic laboratory.

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