
Molecular Typing of Agrobacterium Species Isolates From Catheter-Related Bloodstream Infections
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ABSTRACT

Agrobacterium isolates from intravenous catheters of three hospitalized patients were initially identified as A. tumefaciens, but inability to produce 3-ketolactose revealed that two of them were A. vitis. However, rDNA analysis correlated all of the isolates to A. tumefaciens. Pulsed-field gel electrophoresis analysis ascertained the nosocomial transmission of the infection (Infect Control Hosp Epidemiol 2004;25:885-887).

Bacteria belonging to the genus Agrobacterium are commonly found in soil, but have occasionally been isolated from compromised human hosts with different clinical syndromes. Most of the described cases were patients with bacteremia frequently associated with the presence of an intravascular catheter. Strains isolated from human clinical specimens are usually 3-ketolactose positive and belong to biovar 1, which includes A. tumefaciens, a well-known plant pathogen also used as a gene vector for obtaining genetically modified organisms, and A. radiobacter. Occasionally, 3-ketolactose-negative strains of biovars 2 and 3, now established as separate species and named A. rhizogenes and A. vitis, respectively, have been isolated from clinical specimens but have not been further characterized.

We report the isolation of 3-ketolactose–positive and 3-ketolactose–negative Agrobacterium species bacteria from the blood and intravenous catheters of three patients and describe phenotypic and genotypic characteristics of the isolates.

CASE REPORTS
Case 1
A 58-year-old man was admitted to a coronary intensive care unit for dilatative cardiomyopathy following myocardial infarction. A Hickman central venous catheter (CVC) was inserted at admission. Thirty days later, he developed fever (temperature, 38.2°C) and piperacillin was administered. Peripheral blood for culture was obtained 2 days later and grew A. tumefaciens after 48 hours of incubation. The patient became afebrile 5 days after initiation of therapy, just after his CVC was removed. A. tumefaciens was also isolated from the removed catheter.

Case 2
Two days after the first patient developed bacteremia, a 56-year-old man was admitted for myocardial infarction to the same coronary intensive care unit. This patient also had a Hickman CVC inserted and shared staff with the first patient. The day after admission, he developed fever (temperature, 39°C) and had symptoms of septicemia. Piperacillin was immediately started. Blood cultures were performed 2 and 5 days after admission. Gram-negative bacteria later identified as A. tumefaciens grew from both cultures after 48 to 72 hours of incubation. The patient became afebrile only after 9 days of piperacillin treatment and 3 days after his CVC was removed. A. tumefaciens was also isolated from the catheter.

Case 3
The third patient was a 59-year-old man with a history of remission and relapse of acute lymphocytic leukemia who had a long-term indwelling Hickman CVC for his chemotherapy. After 2 months of weekly controls following antiblastic treatments, the patient presented to the hematology unit with fever (temperature, 39°C) and a white blood cell count of 2,200 mm3 with 38% segmented neutrophils. Blood was collected for culture, his catheter was extracted, and empiric therapy with ciprofloxacin was started. The patient became afebrile after 3 days of antibiotic therapy. The peripheral blood culture and the catheter both grew A. tumefaciens after 48 hours of incubation.

In all cases, A. tumefaciens growth from the catheters was obtained by roll-plating a semiquantitative culture on blood agar. Our isolates were submitted to the Gram-Negative Identification cards with the VITEK 2 system (bioMérieux-Vitek, Hazelwood, MO) for biochemical characteristics and were identified as A. tumefaciens, with a probability of 99%. Most hospital laboratories use phenotypic tests as their only procedure for bacterial identification. Automated systems such as VITEK 2 are increasingly being used for fast and reliable results. However, the VITEK 2 system has never been widely tested for Agrobacterium identification, and it does not include any Agrobacterium species other than A. tumefaciens or A. radiobacter in its 2.01 identification database. Furthermore, some of the characteristics used to discriminate among them (ie, 3-ketolactose production) cannot be tested by this system. When additional phenotypic characteristics were tested (ability to grow at 35°C, ability to grow in the presence of 2% NaCl, production of 3-
ketalactose, and formation of alkali from citrate), the isolates from our first two cases were unable to produce 3-ketalactose and could then be referred to biovar 3 (A. vitis), whereas characteristics of the third isolate corresponded to biovar 1 (A. tumefaciens).

All of the isolates were subjected to further genetic characterization through (1) automatic ribotyping by the Riboprinter microbial characterization system (Qualicon Europe Ltd., Warwick, United Kingdom) with restriction enzyme PstI; (2) restriction analysis of a 4,500-bp polymerase chain reaction amplified fragment of the ribosomal operon (rrn) performed with primers Ad (5’ AGA GTT TGA TCM TGG CTC AG 3’) and O24/3 (5’ CGA CAT CGA GGT GCC AAA 3’) and restriction enzymes HhaI, Ssp3AI, and MboII; and (3) pulsed-field gel electrophoresis analysis of genomic DNA digested with restriction enzymes SpeI, SwaI, and XbaI.

All of the isolates from the three cases showed the same ribotyping pattern. Amplified rrn restriction patterns were identical for the isolates from cases 1 and 2 and similar to those from case 3. With both ribotyping and rrn restriction analysis, profiles of the isolates were genetically close, although not identical, to A. tumefaciens and A. radiobacter reference strains. On the contrary, the type strains of A. vitis, A. rhizogenes, and A. rubi were found to be considerably different from all isolates.

In our study, two patients were in the same intensive care unit, had the same caregivers, and became bacteremic after 2 days. Pulsed-field gel electrophoresis analysis provided evidence for a close relationship between their isolates (Figure). The infection may have been transmitted to the second patient from the one infected first, but a common source of infection for both patients cannot be excluded. Contamination of nurses’ hands and medical equipment or solutions was suspected, but an environmental investigation performed by swab sampling and plate cultures was unable to ascertain the source of the infection. Because the third patient presented with a fever at the hospital 7 days after his last visit, his infection could be considered community acquired, but a healthcare-associated infection cannot be excluded. However, the pulsed-field gel electrophoresis patterns of the third isolate were different enough from those of the previous two to exclude any correlation (Figure).

Our isolates were all susceptible in vitro to ciprofloxacin, which has been suggested as the drug of choice, as well as to pefloxacin, ofloxacin, and norfloxacin. Whereas the third patient with neutropenia had been given ciprofloxacin, the other two patients had been given piperacillin, to which their bacterial isolates were resistant in vitro. In all of the patients, fever decreased only after the removal of the intravenous catheter. Therefore, the removal of foreign bodies could be as important as the effectiveness of the antibiotic therapy for the recovery of the patient.

Revision of bacteriologic analysis data did not reveal any preceding Agrobacterium isolation in the coronary intensive care unit or the hematology unit, and no further cases of infection were registered in the following 24 months after the sanitation of caregivers’ hands and equipment was intensified.

Long-term indwelling venous catheters for administration of parenteral therapy are known to be associated with an increased risk of bloodstream infections. The isolation of Agrobacterium from patients with bacteremia in the presence of an intravascular catheter, although infrequent, is not unusual. An increase in Agrobacterium nosocomial infections can be expected in the future due to the increasing population of immunocompromised and catheterized patients.

On the basis of our data, phenotypic criteria currently adopted to characterize Agrobacterium isolates seem to be inadequate. Specifically, phenotypic characteristics such as 3-ketalactose production might be less discriminating than reported in literature and misleading for identification of Agrobacterium species. Molecular methods based on the analysis of the ribosomal DNA, similar to the ones we used, seem to be suitable to the study of agrobacteria. Their application to the characterization of human isolates can contribute to the management of Agrobacterium infections because a prompt etiologic diagnosis can aid therapeutic decisions. The availability of automatic devices such as the Riboprinter system allows for a wider use of genetic analysis. In hospital units at risk for nosocomial infections, a more precise typing of the isolates, as provided by pulsed-field gel electrophoresis analysis, could help to correctly evaluate nosocomial outbreaks.

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