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Recovery of a Strain of *Agrobacterium radiobacter* with a Mucoid Phenotype from an Immunocompromised Child with Bacteremia

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Agrobacteria are associated more commonly with plant than with human disease. The isolation of *Agrobacterium radiobacter* from blood cultures of an immunocompromised child with a transcutaneous catheter prompted a review of human infections caused by *Agrobacterium* species. Only 12 reports describing 19 cases of *Agrobacterium* infections in humans have appeared in the literature. Sixteen of the patients (84%) were equipped with implantable or transcutaneous medical devices at the time of infection, and 14 of the 19 (80%) patients could be considered immunocompromised because of underlying disease processes. Unlike those in previous reports, however, this patient was infected with a novel mucoid phenotype of *A. radiobacter*. Because of the significant relationship between infection and biomedical implants, we evaluated the adhesion of this mucoid strain and a nonmucoid strain of *A. radiobacter* to plastic by using two in vitro assays. No adhesion or biofilm formation was detected for either strain, but nonetheless it is clear from this review that the isolation of *Agrobacterium* spp. from patients with indwelling medical appliances should not be dismissed as an environmental contaminant.

The *Agrobacterium* genus is most widely recognized as a collection of prominent plant pathogens (11, 12). These organisms are distributed throughout the environment, including soil, and are typically oxidase positive, aerobic, motile, non-spore-forming gram-negative rods that resemble CDC group Vd-3 (19). All *Agrobacterium* species except *A. radiobacter* induce neoplastic growth in a variety of plants after traumatic inoculation into the plant tissue (11, 12). The phytopathogenicity of *Agrobacterium* species is conferred by a 100- to 160-megadalton plasmid called the Ti plasmid (12). Except for the absence of this plasmid, *A. radiobacter* is biochemically indistinguishable from *A. tumefaciens* and, therefore, is likely a single species.

Agrobacteria are infrequently observed in the clinical microbiology laboratory and rarely cause human infection. Prior to this report, only 18 cases of true *Agrobacterium* infection in humans (i.e., excluding the *Agrobacterium* "yellow group") have appeared in the literature. All but two occurred in patients with transcutaneous catheters or implanted biomedical prostheses, and only a single isolate was reported as *Agrobacterium radiobacter* biovar 2 (9). None of the previously reported clinical isolates of *Agrobacterium* species were characterized as mucoid strains. We report here a mucoid phenotype of *Agrobacterium radiobacter* biovar 2 recovered from an immunocompromised child with bacteremia.

A 3½-year-old boy undergoing treatment for a primitive neuroectodermal tumor of the brain was admitted to Texas Children's Hospital in February 1992 for evaluation of fever and sepsis. The child's illness had been diagnosed in March 1990, at which time he underwent partial resection of the tumor followed by chemotherapy, irradiation, and an autologous bone marrow transplantation. A recurrence of the tumor was discovered in September 1991, and he underwent a second resection with placement of a ventriculo-peritoneal shunt, a central venous catheter, and additional therapy with carboplatin and etoposide. In November 1991, the child developed a shunt infection and peritoneal abscess caused by *Streptococcus sanguis* and *Staphylococcus epidermidis* that necessitated drainage of the abscess, shunt removal and replacement, and antimicrobial therapy consisting of vancomycin, amikacin, and ticarcillin for 10 days followed by vancomycin alone for an additional 10 days.

With evidence of progressive disease, his therapy was changed to idarubicin in conjunction with granulocyte colony-stimulating factor in February 1992. On the day prior to admission, the child developed a fever to 39°C and a blood culture was drawn from the central line before an infusion of packed erythrocytes and platelets. The child was not neutropenic at the time, and antimicrobial therapy was not initiated. The following day, gram-negative rods were isolated from both aerobic and anaerobic blood culture bottles (BACTEC Peds Plus and 7A media; Becton Dickinson Diagnostic Instrument Systems, Sparks, Md.) and the child was admitted for further evaluation.

At the time of admission, the child had a temperature of 36.8°C, heart rate of 119, respiratory rate of 28, and a blood pressure of 96/91. The child's lungs were clear to auscultation, and the central line insertion site showed no erythema or drainage. Erythema was observed over the child's face and scalp, but no other rashes or petechiae were noted. The child's leukocyte count at the time of admission was 8×10^9/liter with 5% bands, 43% segmented polymorphonuclear leukocytes, 39% lymphocytes, 11% monocytes, and 2% eosinophils. Four additional blood culture sets (one from each port of the central line and one from a peripheral venous site) were obtained prior to and 1 and 7 days after the administration of antimicrobial therapy consisting of vancomycin (13 mg/kg of body weight every 8 h), gentamicin (2.5 mg/kg every 8 h), and ticarcillin-clavulanate (75 mg/kg every 6 h).

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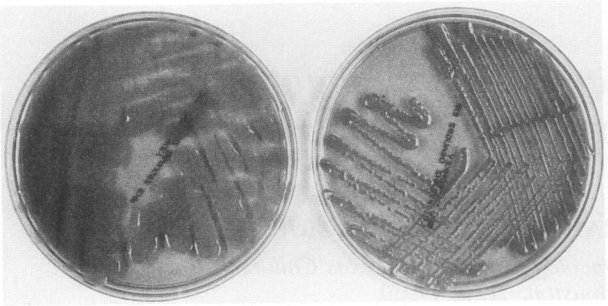

FIG. 1. Comparison of the colonial morphology between mucoid (left) and nonmucoid (right) phenotypes of *A. radiobacter*. Upon continued incubation, the mucoid appearance of the former was gradually lost, suggesting the activation or release of an exopolysaccharide depolymerase.

All blood cultures collected prior to and 1 day after initiating antimicrobial therapy (total of nine) exceeded the positive growth index threshold of the BACTEC 660NR within 24 h of incubation. An oxidase-positive, gram-negative rod was recovered from all positive bottles that produced highly mucoid colonies on MacConkey agar after 3 days of growth at 37°C (Fig. 1). Upon continued incubation, the mucoid appearance of the colonies was gradually lost, suggestive of the activation or release of an exopolysaccharide depolymerase similar to mucoid strains of *Pseudomonas aeruginosa* (6). The organism was identified as *Agrobacterium tumefaciens* (*A. radiobacter*, 99% probability; profile code, 33417021410) by the Vitek AutoMicrobic System (Vitek Systems, Inc., Hazelwood, Mo.). This identification was subsequently duplicated (*A. radiobacter*, 99.8% probability; profile code, 1677744) by the API RapidNFT identification panel (Analytab, Inc., Plainview, N.Y.). The isolate tested positive for oxidase, urease, motility, nitrate reduction (to N_2), and esculin hydrolysis but negative for tryptophan deaminase, arginine dihydrolase, lysine, and ornithine decarboxylase. By the rapid identification panels, the organism utilized the following compounds as sole sources of carbon: maltose, L-arabinose, mannitol, mannose, gluconate, adonitol, xylose, lactose, rhamnose, *N*-acetyl-D-glucosamine, glucose (oxidatively), and malate. It did not utilize inositol, raffinose, sorbitol, glucose (fermentatively), malonate, or sucrose. However, slow sucrose utilization was demonstrated with rapid fermentation medium (BBL Microbiology Systems, Cockeysville, Md.). The organism was further identified as *A. radiobacter* biovar 2 on the basis of a negative assay for 3-ketolactose production on lactose-containing medium (2) and the absence of a demonstrable Ti plasmid by using the method of Portnoy and Falkow (16) with *A. tumefaciens* (ATCC 15955) as a positive control. The identification of this isolate was confirmed by the Texas State Health Department Microbiology Laboratory by cellular fatty acid analysis. Broth macrodilution susceptibility testing of the isolate with cation-supplemented Mueller-Hinton broth (14) demonstrated the following results: amikacin MIC = 8 μg/ml, MBC = 16 μg/ml; gentamicin MIC = 4 μg/ml, MBC = 8 μg/ml; trimethoprim and sulfamethoxazole MICs = 0.03 and 0.6 μg/ml, respectively, MBC = 4 and 80 μg/ml, respectively; ampicillin MIC = MBC = 1 μg/ml; cephalothin MIC = 2 μg/ml, MBC = 8 μg/ml; cefotaxime MIC = MBC = 0.5 μg/ml; ceftazidime MIC = 8 μg/ml, MBC = 32 μg/ml; and piperacillin MIC = 8 μg/ml, MBC = 16 μg/ml. By the Vitek System, the organism was also shown to be susceptible to ticarcillin (≤16 μg/ml), imipenem (≤4 μg/ml), and ciprofloxacin (≤0.5 μg/ml) but resistant to aztreonam and chloramphenicol (≥32 μg/ml) by criteria for oxidase-positive organisms other than pseudomonads. On the basis of these findings and an uncomplicated course, vancomycin was discontinued from the therapeutic regimen and the child was discharged on the 8th hospital day but continued to receive parenteral antimicrobial therapy for 10 additional days.

To address the apparent association between infections caused by agrobacteria and biomedical implants, we evaluated the adhesive qualities of the mucoid strain and a nonmucoid strain of *A. radiobacter* biovar 1 recovered from another source by using an in vitro model of adhesion (7) and biofilm formation (5). No adhesion to or biofilm formation over the surface of polystyrene microtiter plates was de-

TABLE 1. Summary of reported cases of human *Agrobacterium* infection

Patient	Yr reported (reference)	Type of implant	*Agrobacterium* species	Source	Underlying disease
1	1980 (15)	Heart valve	*A. radiobacter*	Blood	Endocarditis
2	1985 (1)	Nephrostomy tube	*A. radiobacter* biovar 1	Urine	Prostatic adenoma
3	1985 (9)	Intravascular catheter	*A. radiobacter* biovar 2	Blood	Acute pneumonia
4	1987 (4)	None	*A. radiobacter*	Blood	Cholecystitis
5	1987 (8)	Central venous catheter	*A. radiobacter* biovar 1	Blood	Adenocarcinoma
6	1988 (22)	Hickman catheter	*A. radiobacter* biovar 1	Blood	Myeloid leukemia
7	1989 (17)	Central venous catheter	*A. radiobacter* biovar 1	Blood	Hirschsprung's
8	1989 (3)	Central venous catheter	*A. radiobacter*	Blood	Aplastic anemia
9	1989 (3)	Central venous catheter	*A. radiobacter*	Blood	Adenocarcinoma
10	1991 (21)	Central venous catheter	*A. radiobacter*	Blood	Human immunodeficiency virus infection
11	1991 (21)	Central venous catheter	*A. radiobacter*	Blood	Lymphoma
12	1991 (21)	Central venous catheter	*A. radiobacter*	Blood	Leukemia
13	1991 (21)	Peritoneal catheter	*A. radiobacter*	Peritoneal dialysate	Ovarian carcinoma
14	1991 (21)	Nephrostomy tube	*A. radiobacter*	Urine	Ovarian carcinoma
15	1991 (10)	Central venous catheter	*A. radiobacter* biovar 1	Blood	Leukemia
16–17	1991 (20)	Peritoneal catheter	*A. radiobacter*	Peritoneal dialysate	Renal failure
18	1992 (18)	Not indicated	*A. tumefaciens*	Liver and peritoneal biopsy	Cirrhosis and portal hypertension
19	1992 (This study)	Central venous catheter	*A. radiobacter* biovar 2	Blood	Neuroectodermal tumor

tected for either strain regardless of incubation temperature (22, 30, or 37°C), growth medium (Trypticase soy or Mueller-Hinton broth), or duration of incubation (up to 7 days) or after supplementation of the growth medium with 10% serum protein. Furthermore, no bacterial adhesion to plastic microwells was detected after the wells had been coated overnight with 10% fetal calf serum.

Prior to the report of Plotkin (15) in 1980, the isolation of *Agrobacterium* species from clinical sources was considered incidental and a likely indication of environmental contamination (13, 19). Since then, 13 reports (including the present one) describing 19 cases of *Agrobacterium* infection in humans have appeared in the literature (Table 1). Of these, the source of the organism was blood in 13 patients, peritoneal dialysate in 3 patients, urine in 2 patients, and liver-peritoneal biopsy in 1 patient. Seventeen of the 19 patients had implanted or transcutaneous biomedical devices at the time of the infection, and at least 14 of the patients were immunocompromised because of a primary disease process. The results of antimicrobial susceptibility testing of clinical isolates reported to date have varied widely and provide no predictable resistance profile. However, none of the reported cases proved to be fatal, indicating that *Agrobacterium* species are not particularly virulent.

On the basis of a review of the literature, we believe this case represents the second report of human infection caused by *A. radiobacter* biovar 2 and the first description of a mucoid phenotype of *A. radiobacter*. According to Kersters and De Ley (12), *Agrobacterium* biovar 2 strains are incapable of growth at 35°C. However, this and the previous clinical isolate of *Agrobacterium* biovar 2 (9) demonstrated good growth at 35°C, suggesting thermal adaptation during the course of human infection. Although we were unable to demonstrate in vitro adhesion to plastic, the presence of a biomedical implant and/or immunosuppression appears to be a strong risk factor for the development of *Agrobacterium* infection in humans. The nature of the mucoid material is currently unknown, but studies have been initiated to determine its chemical composition.

Similar to coagulase-negative staphylococci, the prevalent use of implantable medical devices and immunosuppressive therapies will likely increase the frequency of isolation of *A. tumefaciens* and *A. radiobacter* in the clinical microbiology laboratory.

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