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Curtobacterium flaccumfaciens Septic Arthritis following Puncture with a Coxspur Hawthorn Thorn▽

Michelle J. Francis,[1]* Richard R. Doherty,[2,6] Minoo Patel,[3] John F. Hamblin,[1] Samar Ojaimi,[4] and Tony M. Korman[1,4,5]

Departments of Microbiology,[1] Paediatric Infectious Diseases,[2] Orthopaedic Surgery,[3] and Infectious Diseases,[4] Monash Medical Centre, 246 Clayton Road, Clayton, Victoria 3168, Australia, and Departments of Medicine[5] and Paediatrics,[6] Monash University, Clayton, Victoria 3168, Australia

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***Curtobacterium* species are recognized plant pathogens. We report the first well-documented case of *Curtobacterium* human infection, a child with septic arthritis following puncture with a Coxspur Hawthorn plant thorn. The organism isolated from synovial tissue and the plant thorn was identified as *Curtobacterium flaccumfaciens* by 16S rRNA gene sequence analysis.**

CASE REPORT

A previously healthy 7-year-old boy presented with right knee pain 1 day after being pushed into a thorny bush at school. He had noticed swelling and redness at the site of the puncture and was unable to bear weight on his right leg. His symptoms settled somewhat with analgesia and immobilization, and he was discharged the following day. He returned 2 days later with increasing right knee pain, swelling, and fever. He was discharged the next day without antibiotic therapy.

Five days later (10 days after the injury), he presented again with a limp and ongoing fevers of up to 39°C. On examination, there was reduced range of right knee flexion, knee swelling without erythema, and a joint effusion. There was a healing puncture wound on the lateral aspect, and palpation of the surrounding area did not suggest the presence of a foreign body. The peripheral blood leukocyte count was 13.1×10^9 cells/liter (77% neutrophils). He underwent an arthroscopic washout and synovial biopsy of the right knee. A 1-cm-long piece of thorn was identified in the synovial tissue and removed. Histological examination was consistent with acute synovitis and septic arthritis.

Perioperative intravenous (i.v.) cefazolin was administered for 24 h and then changed to i.v. ticarcillin-clavulanate for 8 days. He remained afebrile from the second day of admission and was discharged to continue oral amoxicillin (80 mg/kg body weight/day) for a further 4 weeks. The residual knee swelling and stiffness resolved slowly with physical therapies over the weeks after discharge. At the 6-month follow-up, he had made a full recovery, with no limitation of movement in the affected knee.

Microbiology. The Gram stain of the synovial fluid and knee tissue revealed numerous polymorphonuclear leukocytes but no bacteria. In aerobic conditions at 35°C, bacterial growth was observed on horse blood agar at 24 h. The organism grew equally well and with identical morphology at both 30°C and 37°C. The colonies were nonhemolytic, smooth, entire, low convex, with a yellow pigment. Colonies were 1 to 1.5 mm in diameter after 48 h incubation. Gram stain showed Gram-positive small, short rods, some in palisade arrangements, with no branching. The isolate was nonmotile, catalase positive, and urease negative and strongly hydrolyzed esculin. The API Coryne version 3.0 identification system (bioMérieux, Marcy l'Etoile, France) gave an identification of *Brevibacterium* species, with a confidence value of 79% (code 2040005). MICs determined using Etest (bioMérieux, Marcy l'Etoile, France) were 0.125 μg/ml for penicillin and 0.25 μg/ml for cefotaxime and vancomycin.

Because of the inconclusive phenotypic identification, molecular identification was performed using the MicroSEQ500 16S rDNA bacterial identification kit (Perkin-Elmer, Applied Biosystems, Foster City, CA). Sequence analysis performed with 467 nucleotides using MicroSEQ 500 software (version 1.2) demonstrated 99.89% sequence homology with *Curtobacterium flaccumfaciens* (CCM 1587). Sequence analysis with BLAST version 2.0 (http://www.ncbi.nlm.nih.gov/BLAST) revealed 100% identity with *C. flaccumfaciens* pv. flaccumfaciens (DSM 20129).

A branch of the plant source of the thorn was retrieved by the patient's mother and identified as coxspur hawthorn (*Crataegus crus-galli*). A leaf and thorn from the coxspur hawthorn plant were separately cultured on horse blood agar. From the leaf, large, gray, nonhemolytic colonies were isolated. Gram stain demonstrated a Gram-negative bacillus which was identified by 16S rRNA sequencing as *Pantoea agglomerans* (99.38%). From the thorn, an organism phenotypically similar to that isolated from the knee tissue at operation was isolated and was confirmed as *C. flaccumfaciens* using 16S rRNA sequencing.

Discussion. The genus *Curtobacterium* was proposed by Yamada and Komagata in 1972 for some so-called motile brevibacteria and plant pathogens previously classified as corynebacteria (11). Curtobacteria are obligate aerobic Gram-positive coryneform bacteria with a group B-type peptidoglycan structure markedly different from that of true corynebacteria and characteristic cell wall lipid composition (2).

The *Curtobacterium* genus is a member of the family *Microbacteriaceae* and contains well-established species, including *C.*

* Corresponding author. Mailing address: Department of Microbiology, Monash Medical Centre, 246 Clayton Rd., Clayton, Victoria 3168, Australia. Phone: 61 3 95944532. Fax: 61 3 95946223. E-mail: michelle.francis@southernhealth.org.au.

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albidum, *C. citreum*, *C. flaccumfaciens*, *C. herbarum*, *C. luteum*, and *C. pusillum* (9). Further species *C. ammoniigenes* and *C. ginsengisoli* have been recently proposed (1, 8). The organism named *C. plantarum* may not actually be a member of the genus (5). The main natural habitats of *Curtobacterium* species are different plants, although they also occur in soil and other environments (4). *C. citreum*, *C. albidum*, and *C. luteum* are not known to cause any disease on rice, from which they were primarily isolated (9).

C. flaccumfaciens is a well-established plant pathogen (4). *C. flaccumfaciens* is divided into four pathovars for epidemiological purposes. *C. flaccumfaciens* pv. flaccumfaciens causes the wilt of beans, *C. flaccumfaciens* pv. betae infects red beet, inducing wilt and silvering of leaves, while *C. flaccumfaciens* pv. oortii is a causative agent of wilt and spots of the leaves and bulbs of tulips, and *C. flaccumfaciens* pv. poinsettiae causes stem canker and leaf spots of poinsettia. It has also been isolated from the leaves and seeds of a variety of species of herbs, shrubs, and trees, where it is considered to be a saprophyte (3, 4, 10). The occurrence of *C. flaccumfaciens* has been recorded in diverse geographical areas within Europe, Australia, Asia, North and South America, and Africa (7).

The yellow-pigmented Gram-positive bacillus isolated from the synovial tissue and the plant thorn grew equally at 30°C and 37°C. This was consistent with *C. flaccumfaciens* pv. flaccumfaciens, as all other *Curtobacterium* species show better growth at 30°C than at 37°C (4, 5, 6, 9). The strain was nonmotile, whereas most *Curtobacterium* strains were motile in other reports (5).

Typical phenotypic characteristics of *C. flaccumfaciens* included positive catalase, negative urease, and esculin hydrolysis. Acid production from carbohydrates by curtobacteria is very slow (up to 7 days or longer) compared to that of microbacteria (1 to 2 days).

Curtobacterial strains may not be recognized by clinical laboratories due to optimal growth at 30°C and because they are not included in the databases of commercial identification systems. Specialist chemotaxonomic investigations (analysis of cellular fatty acids, cell wall components, and peptidoglycan structure) are necessary for definitive phenotypic identification of the organisms. Although these may be reasons for underdiagnosis, experience from reference laboratories suggests that curtobacteria are actually the least frequently encountered yellow- or orange-pigmented coryneform bacteria in clinical specimens (5).

Molecular techniques are now more readily available to clinical laboratories and may assist in the identification of curtobacteria. The 16S rRNA gene sequence of the strains isolated from synovial tissue and the plant thorn had 100% identity with *C. flaccumfaciens.*

Funke et al. (5) reported the only five *Curtobacterium* strains recovered from human clinical specimens. In three cases, a disease association was suggested by heavy growth in pure culture with a moderate leukocyte reaction on direct Gram stains of the clinical material (wound, eye discharge, and lymph node tissue). In two other cases, a disease association was not so clear, as the two strains were isolated from sputum in mixed culture, although they were the predominant microorganisms. The 16S rRNA gene sequence of two strains had 100% homology with *C. flaccumfaciens.*

In our case, the strain had a low penicillin MIC, and the patient responded to beta-lactam therapy. Funke et al. reported antimicrobial susceptibilities for 15 *Curtobacterium* isolates. MICs of teicoplanin and vancomycin were lower than 2 μg/ml for all strains (5). The 50% and 90% MICs of beta-lactams were mostly greater than 1 μg/ml. Macrolides and rifampin showed very low MICs for all strains tested.

We believe that this report of septic arthritis following puncture with a coxspur hawthorn thorn is the first well-documented clinical case of *Curtobacterium* causing infection in humans. *C. flaccumfaciens* was isolated from synovial tissue and the plant thorn and identified by 16S rRNA gene sequence analysis. *Curtobacterium* should be considered a cause of plant-associated bacterial disease in humans. The use of molecular techniques allows clinical microbiology laboratories to confirm the identity of bacterial species which have not been previously described as causes of human disease.

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The authors report no conflicts of interest.

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