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NOTES

Case of Fatal Systemic Infection with an *Aureobacterium* sp.: Identification of Isolate by 16S rRNA Gene Analysis

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The case of a 75-year-old man who succumbed to a disseminated infection most likely caused by a species of the genus *Aureobacterium* is reported. Identification of the isolate was achieved by comparative 16S rRNA gene analysis. Aureobacteria are commonly found in the environment. However, only recently have they been recognized as a cause of infections including septicemia and soft tissue infections. To our knowledge, this is the first documentation of a fatal infection caused by an *Aureobacterium* sp.

A 75-year-old man was hospitalized because of severe abdominal pain. The symptoms had reportedly commenced about 24 h prior to admission with sudden chills, sweating, and a slight pain in his limbs. During the first day at the hospital he developed severe myalgia, arthralgia, and oliguria, but no fever. By day 4 he suffered anuric kidney failure and hemofiltration was started. On the same day the body temperature suddenly rose to 42°C and disseminated intravascular coagulation and generalized convulsions occurred. Blood samples for culture were taken and antibiotic therapy with imipenem, penicillin G, and ciprofloxacin was started. In addition, the patient received 7S immunoglobulins. Despite artificial respiration, maximum supportive measures, and antibiotic treatment, the patient died on day 5.

Autopsy revealed focal granulocytic infiltration of the kidneys, liver, and spleen. Diffuse edema and multiple hemorrhagic foci were found in all parts of the brain. The mucous membranes of the colon as well as parts of the small intestine showed massive hemorrhage, and signs of terminal cardiopulmonary failure were evident.

Small, gram-positive, irregular rods were detected in Gram-stained samples taken from the meninges, kidneys, liver, and spleen. Subsequently, gram-positive rods were recovered from the same samples on blood agar under aerobic conditions. The colonies displayed a bright yellow pigmentation and tested catalase positive. The blood culture taken 1 day before the patient died yielded an organism with an appearance identical to those of the organisms in cultures of tissue samples. This confirmed the clinical significance of the finding and virtually excluded the possibility that the postmortem isolate represented a contaminant. Attempts to classify the organism by conventional biochemical tests (API CORYNE, RAPID ID32 A, and Rap ANA II systems) failed. A laboratory specializing in the diagnostics of gram-positive rods identified the isolate as a *Cellulomonas* sp.

Since members of the genus *Cellulomonas* are not usual pathogens, we sought to confirm the diagnosis independently. We amplified and sequenced most of the gene encoding the 16S rRNA in order to perform a phylogenetic analysis. Genomic DNA was extracted as described elsewhere (4). Following amplification of a 1.5-kbp fragment with conserved primers, two independent approaches were used. First, cloned subfragments of about 650 and 750 bp were sequenced by using digoxigenin-labelled primers and an electrophoresis and blotting device (4). Second, automated *Taq* dye terminator sequencing of the PCR product was performed on a 373A Applied Biosystems instrument. The amplification primers were GAGAGTTTGATCCTGGCTCAG (forward) and AAGGAG GTGATCCAGCCGCA (reverse). The sequencing primers were AACACATGCAAGTCGAACG, CTACGGGAGGCAGCAG TGGG, CAGCAGCCGCGGTAATAC, AAACTCAAAGGAA TTGACGG, CATGGCTGTCGTCAGCTCGT, and ACGGGC GGTGTGTAC for the upper strand and CCCACTGCTGCCT CCCGTAG, GTATTACCGCGGCTGCTG, CCGTCAATTCC TTTGAGTTT, ACGAGCTGACGACAGCCATG, and ACG GGCGGTGTGTAC for the lower strand. The resulting sequence was added to an alignment of about 2,000 homologous sequences from bacteria (5). Maximum likelihood analysis (fast DNAm1) showed the isolate to be closely related to various strains of the genus *Aureobacterium* (Fig. 1).

The genus name *Aureobacterium* was first introduced for coryneform bacteria that share a number of specific biochemical features including peptidoglycan type, G+C content, and menaquinone patterns (2). In addition, some species originally grouped within other genera were recently proposed to be transferred to the genus *Aureobacterium* on the basis of molecular data (6).

Data concerning the clinical significance of *Aureobacterium* spp. are scarce. In fact, we are aware of only two studies dealing with clinical isolates classified as *Aureobacterium* spp. According to both reports, the clinical *Aureobacterium* isolates were initially incorrectly identified as *Corynebacterium aquaticum* and *Brevibacterium* spp., respectively (1, 3). These *Aureobacterium* spp. had been isolated from blood cultures, cerebrospinal fluid, an epidural abscess, soft tissue, and intra-abdominal swabs, indicating that *Aureobacterium* spp. may cause invasive disease. However, these infections were reportedly self-limited (1). In contrast, the present case of infection took a fulminant course, leading to death within 5 days after manifestation of the first symptoms and only 1 day after the onset of fever. It remains unclear whether a specific condition of the deceased patient, the properties of the isolated organism, or additional factors (e.g., a second, unrecognized patho-

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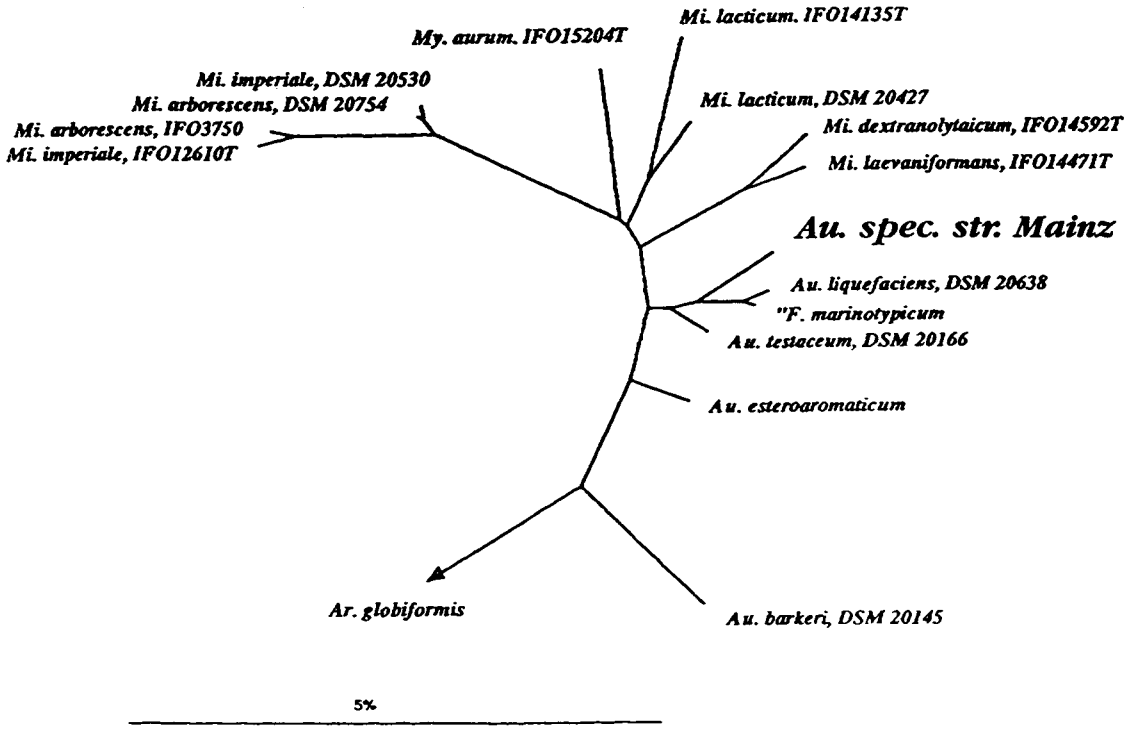

FIG. 1. Maximum likelihood tree reflecting the phylogenetic relationship of *Aureobacterium* sp. strain Mainz and other species of the genus and some related strains. The length bar indicates 5% estimated sequence divergence. *Arthrobacter globiformis* served as the outgroup. *Mi.*, *Microbacterium*; *My.*, *Mycobacterium.*

gen) or a combination of such factors were responsible for the fatal outcome. The source of infection with an *Aureobacterium* sp. remains speculative. However, it seems likely that the patient acquired the infection through skin abrasions on his forearms while working with soil and sand at his weekend hut in the forest 1 week before he fell ill. Alternatively, an insect bite on his knee, possibly caused by a tick, may have served as a port of entry for the pathogen. The patient had earlier been vaccinated against spring-summer meningoencephalitis. Tick-borne rickettsial diseases are not endemic in middle Europe, and they are usually accompanied by a rash, which was not observed in our patient.

A broad range of serological tests was performed; moderate titers of immunoglobulin G against *Treponema pallidum* and against hepatitis B virus core antigen indicated earlier infection with these pathogens. No evidence of any other acute or chronic infection or an immunodeficiency was found. The patient's age and a severe arteriosclerosis may have contributed to the dramatic course of the infection. However, we could not identify a typical risk factor that would satisfactorily explain why this patient died from an infection with an opportunistic pathogen. The wide dissemination of the bacterium suggests a gross failure of defense mechanisms rather than an escape from phagocytosis of a few cells. Possibly, prevention of access to sterile compartments represents the major protective factor against infection with certain environmental bacteria such as *Aureobacterium* spp.

In vitro susceptibility testing of the isolate suggested that penicillin should have been effective. However, the lack of fever during the early stages of the disease concealed the septic nature of the ongoing process so that antibiotic therapy was probably initiated too late. A detrimental antibiotic effect akin to a Herxheimer reaction cannot be excluded.

The two recent reports of clinical *Aureobacterium* isolates as well as the present report demonstrate the difficulties that may be encountered with the identification of some gram-positive rods by conventional methods. Even the laborious analysis of peptidoglycan type does not necessarily lead to a reliable species identification. Analysis of the 16S rRNA gene probably is the most practical approach to identifying this potential pathogen in the clinical microbiology laboratory.

The strain described here has been deposited with the Culture Collection, University of Göteborg, Göteborg, Sweden, as CCUG 35327. The 16S rRNA gene sequence is available from EMBL under accession number X93589.

(The work was done in partial fulfillment of the doctoral thesis of A. Feddersen.)

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REFERENCES

1. **Carlotti, A., H. Meugnier, M. T. Pommier, J. Villard, and J. Freney.** 1993. Chemotaxonomy and molecular taxonomy of some coryneform clinical isolates. Int. J. Med. Microbiol. Virol. Parasitol. Infect. Dis. **278:**23–33.
2. **Collins, M., D. Jones, R. M. Keddie, R. M. Kroppenstedt, and K. H. Schleifer.** 1983. Classification of some coryneform bacteria in a new genus *Aureobacterium*. Syst. Appl. Microbiol. **4:**236–252.
3. **Funke, G., A. von Graevenitz, and N. Weiss.** 1994. Primary identification of *Aureobacterium* spp. isolated from clinical specimens as "*Corynebacterium aquaticum*." J. Clin. Microbiol. **32:**2686–2691.
4. **Husmann, M., C. Gries, P. Jehnichen, T. Woelfel, G. Gerken, W. Ludwig, and S. Bhakdi.** 1994. *Helicobacter* sp. strain *Mainz* isolated from an AIDS patient with septic arthritis: case report and nonradioactive analysis of 16S rRNA sequence. J. Clin. Microbiol. **32:**3037–3039.
5. **Strunk, O., et al.** Unpublished data.
6. **Yokota, A., M. Takeuchi, T. Sakane, and N. Weiss.** 1993. Proposal of six new species in the genus *Aureobacterium* and transfer of *Flavobacterium esteromaticum* Omelianski to the genus *Aureobacterium* as *Aureobacterium esteromaticum* comb. nov. Int. J. Syst. Bacteriol. **43:**555–564.