We report a case of endocarditis due to *Arthrobacter woluwensis* and review the published reports of *Arthrobacter* species isolated from human clinical samples. A 39-year-old injection drug user presented with fever and a new heart murmur. *A. woluwensis* was isolated from blood cultures, and a diagnosis of subacute infective endocarditis of the native mitral valve was made. The patient was successfully treated with a 6-week course of intravenous teicoplanin. From our review of the literature, we were able to retrieve data on 41 cases of *Arthrobacter* species isolated from human clinical samples. However, *Arthrobacter* species was documented as a cause of human disease on only 5 other occasions (2 cases of bacteremia, 1 case of postoperative endophthalmitis, 1 case of a Whipple disease–like syndrome, and 1 case of phlebitis). Because of the difficulty of identifying *Arthrobacter* strains by conventional biochemical assays, it is likely that infections with these coryneform bacteria are underreported.

Coryneform bacteria, usually considered part of the normal human flora or environmental contaminants, have increasingly been recognized as a cause of life-threatening diseases. In the general population, coryneform bacteria are the agents in ~1% of cases of infective endocarditis on both prosthetic and native valves [1] but in up to 7% of cases of early-onset prosthetic valve endocarditis [2]. The identification of coryneform bacteria to the genus and species level remains a challenge for most laboratories dealing with human clinical samples. Despite the wide distribution of these species in the environment, especially in soil, the potential for *Arthrobacter* species, which belong to the coryneform bacteria, to cause human disease has been fully recognized only in recent years [3].

We report a case of subacute infective endocarditis with involvement of the native mitral valve due to *Arthrobacter woluwensis* in a HIV-seronegative injection drug user. In addition, we review the published reports of *Arthrobacter* species recovered from human clinical samples since the first description of 11 clinical strains belonging to this genus in 1996.

**PATIENTS, MATERIALS, AND METHODS**

**Patients.** In addition to our case, previously reported cases involving the isolation of *Arthrobacter* species from clinical specimens were identified by means of a MEDLINE search covering literature from the year of the first isolation of *Arthrobacter* from clinical specimens (1996) through May 2002, with “Arthrobacter” used as the keyword. The reference sections of these publications were used to check whether the MEDLINE search was complete.

**Conventional microbiological assays.** The prelim-
inary biochemical characterization of the gram-positive rods isolated from the patient’s blood specimens was performed by using the API Coryne system, database version 2.0 (bio-Mérieux). Additional analyses were performed according to the identification system for coryneform bacteria established by Funke and Bernard [4].

Assays for molecular analysis. DNA was extracted from single colonies recovered from Columbia agar in 200 μL of a commercial ion-exchange resin (InstaGene matrix; Bio-Rad), in accordance with the manufacturer’s instructions. A standard PCR reaction was performed with 5 μL of the extracted DNA as template, 0.5 μmol/L of each primer, and 1 U Taq polymerase (Qiagen) in a total reaction volume of 50 μL (buffer was provided by the manufacturer). The reactions were overlaid with paraffin oil (Merck) to prevent evaporation. Universal PCR primers amplifying the whole ribosomal 16S rDNA gene were UNI16SRNA-L (5′-ATTCTAGAGTTTGATCATGGCTCA-3′) and UNI16SRNA-r (5′-ATGTTACCGTGTGACGGGCGGTGTGTTGA-3′). PCR was performed in a DNA Thermal Cycler (Perkin-Elmer Applied Biosystems) under the following conditions: denaturation at 94°C for 30 s, annealing at 52°C for 30 s, and elongation at 72°C for 60 s, for a total of 35 cycles. The amplification products were used as templates for direct sequencing, prepared by a simple purification step with the QIAquick PCR Purification Kit (Qiagen), in accordance with the manufacturer’s instructions. Cycle sequencing reactions were performed in total volumes of 15 μL with an ABI Prism Big Dye Terminator Cycle Sequencing Kit (Perkin-Elmer) on an ABI Prism 310 Genetic Analyzer (Perkin-Elmer) under the following conditions: 96°C for 30 s, and 30 cycles of 5 s at 50°C, 10 s at 60°C, and 5 s at 96°C. All sequences were compared with data deposited in GenBank; the results were identical.

CASE REPORT

A 39-year-old injection drug user was first seen because of remittent fever with chills, fatigue, myalgia, and a weight loss of 7 kg in the previous 6 months. Initially, he refused to be hospitalized and could not be traced for the next 7 weeks. Thereafter, he presented to our emergency department because of persistent fever and increasing weakness. He had been a known addict to injected heroin, triturated amphetamines, and cocaine for almost 15 years. He had pulmonary tuberculosis during childhood and adolescence, experienced episodes of generalized convulsions at the age of 28 years, received a diagnosis of sacralilitis on the right side 5 years before admission, and underwent surgical excision of a right iliopsoas abscess 1 year before admission. However, there was no history of heart or rheumatic disease. He had repeatedly negative HIV test results in the past 2 years.

At admission, his axillary temperature was 38.5°C, his pulse was 60 beats per minute, and his respiratory rate was 15 breaths per minute. Blood pressure was 90/60 mm Hg. His height was 164 cm, and his body weight was 53 kg. The relevant clinical findings were a new mitral regurgitation murmur, a large hernia at the right iliac wound, and a palpable spleen. The lungs were clear on auscultation. There were no abnormalities of the ocular fundi, no skin lesions, and no neurological abnormalities. Laboratory tests revealed normochromic anemia (hemoglobin level, 10.8 g/dL), a WBC count of 4.3 × 10⁷ cells/L (with 91% neutrophils), and a C-reactive protein level of 60 mg/L. Lactate dehydrogenase level, creatinine level, alanine aminotransferase level, alkaline phosphatase level, prothrombin time, partial thromboplastin time, and urine sediment level were normal. The electrocardiogram and the radiograph of the chest revealed no abnormalities. Abdominal ultrasound revealed a slightly enlarged steatotic liver and a homogenous spleen with a maximum diameter of 13 cm.

Four blood culture sets (4 bottles each visit) were taken and incubated in a BacT/ALERT system (bioMérieux). The report from the first culture indicated only presence of gram-positive rods; the report of the other blood culture sets revealed the presence of a presumed strain of Corynebacterium aquaticum (growth only in the aerobic blood culture bottles). Identification was performed on the basis of Gram stain, morphology of the colonies, and biochemical reaction, as assessed by a commercial system (see Results). The organism was resistant to penicillin and ciprofloxacin but susceptible to tetracycline, vancomycin, and teicoplanin by the disk diffusion assay. Trans-thoracic and transthoracic echocardiography revealed no vegetations, but modest mitral regurgitation and minimal aortic regurgitation were present. The patient received a diagnosis of subacute infective endocarditis on the basis of the revised criteria of Duke University [5].

The patient was provided intravenous teicoplanin (400 mg b.i.d.) for 6 weeks. His fever subsided after the first 24 h of treatment, and the C-reactive protein level normalized after 11 days. The subsequent clinical course was uneventful. The results of cultures of blood samples obtained 3 weeks after the start of antibiotic therapy and at the end of treatment remained negative. Three months after the end of treatment, the cardiac murmur had almost disappeared, and transthoracic echocardiography revealed no additional abnormalities.

RESULTS

First isolation and identification as C. aquaticum. Standard blood culture with the Bactalert culture system and Organon
Table 1. Clinical features of patients with infection due to *Arthrobacter* species.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Age in years, sex</th>
<th>Clinical findings</th>
<th>Diagnosis</th>
<th>Predisposing condition or risk factor</th>
<th>Species identified</th>
<th>Treatment (duration, weeks)</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>[17]</td>
<td>72, F</td>
<td>Ocular pain, loss of visual acuity, hypopyon</td>
<td>Postoperative endophthalmitis</td>
<td>Intraocular lens implantation</td>
<td><em>Arthrobacter</em> species</td>
<td>IV, intravitreal, and topical vancomycin and gentamicin, followed by oral amoxicillin (unknown)</td>
<td>Cured</td>
</tr>
<tr>
<td>[3]</td>
<td>33, F</td>
<td>Fever, bacteremia (5 positive blood culture results)</td>
<td>Port-a-Cath (SIMS Deltec) infection (clinical suspicion)</td>
<td>AIDS</td>
<td><em>Arthrobacter woluwensis</em></td>
<td>IV ampicillin (2)</td>
<td>Cured</td>
</tr>
<tr>
<td>[18]</td>
<td>60, M</td>
<td>Fever, asymmetric polyarthritis, buccal aphthous ulcerations, loss of visual acuity</td>
<td>Whipple syndrome (i.e., uveitis, B27-negative spondylarthropathy, meningitis, lymphadenopathy)</td>
<td>None</td>
<td><em>Arthrobacter</em> species&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Oral TMP-SMZ and rifampin (unknown)</td>
<td>Cured</td>
</tr>
<tr>
<td>[8]</td>
<td>Unknown</td>
<td>Unknown</td>
<td>Severe phlebitis</td>
<td>Previous surgical intervention</td>
<td><em>Arthrobacter albus</em></td>
<td>Unknown</td>
<td>NA</td>
</tr>
<tr>
<td>PR</td>
<td>39, M</td>
<td>Fever, new-onset heart murmur, bacteremia (4 positive blood culture results)</td>
<td>Endocarditis</td>
<td>Injection drug use</td>
<td><em>A. woluwensis</em></td>
<td>IV teicoplanin (6)</td>
<td>Cured</td>
</tr>
</tbody>
</table>

**NOTE.** PR, present report; TMP-SMZ, trimethoprim-sulfamethoxazole.

<sup>a</sup> Molecular analysis based on 16S rRNA gene amplification applied to pretreatment biopsy specimens of inguinal lymph node.
Teknika bottles revealed the presence of gram-positive rods. After subculturing on Columbia blood agar and chocolate agar plates, the microorganisms grew at 37°C within 24 h. Biochemical characterization by the use of the API Coryne system resulted in a species identification as *C. aquaticum* with a probability of 99.9% and T of 0.87 (internal identification parameters of the API software ATB Plus [bioMérieux]). The macroscopic appearance of the colonies (initially whitish-grayish and becoming slightly yellowish after 72 h), as well as the size of the smooth colonies (diameter, >2 mm) after only 24 h incubation at 37°C in a 5% CO2-enriched atmosphere, made assignment to the genus *Corynebacterium* most unlikely, because true corynebacteria very rarely exhibit a yellowish pigment and are hardly ever larger than 2 mm in diameter after only 24 h. These features prompted us to study the isolates in more detail.

**Identification as *A. woluwensis***. We observed the following reactions: catalase, positive; oxidative metabolism, motility, negative; NO2 reduction, negative; urea hydrolysis, positive; esculin hydrolysis, positive; no acid production from glucose; maltose; sucrose; mannitol; xylose; cAMP reaction, negative; DNase activity, positive; and gelatinase, positive; and no lipopolysaccharide. Analysis of cellular fatty acids revealed C15:0 i (53% of total cellular fatty acids), C17:0 i (20%), C15:0 i (9%), and C16:0 i (8%) as the predominant cellular fatty acids. In addition, we determined using chemotaxonomic methods [3] that lysine was the diamino acid of the peptidoglycan. The results of these investigations demonstrated conclusively that the unknown bacterium belonged to the genus *Arthrobacter* [6]. Finally, we compared the unknown bacterium with the type strain of *A. woluwensis* (strain DSM 10495) and observed that both strains were able to hydrolyze starch, casein, and tyrosine but not xanthine.

The sequence of the DNA obtained from the patient’s blood culture matched a sequence deposited in GenBank (accession number X93353) that is associated with *A. woluwensis*. The GenBank accession number assigned to the sequence is AJ112986.

**Antimicrobial susceptibilities**. The antimicrobial susceptibility pattern of the patient’s strain was determined as described elsewhere [7], and the following MICs were observed: penicillin, 4 mg/L; ampicillin, 8 mg/L; cefuroxime, >64 mg/L; cephalothin, 64 mg/L; ceftriaxone, 64 mg/L; chloramphenicol, 1 mg/L; tetracycline, 1 mg/L; ciprofloxacin, 4 mg/L; clindamycin, 2 mg/L; gentamicin, 8 mg/L; rifampin, <0.125 mg/L; teicoplanin, 0.125 mg/L; and vancomycin, 2 mg/L. Except for rifampin, these values were in concordance with data described for the *A. woluwensis* type strain [3].

**DISCUSSION**

Only recently have isolates of *Arthrobacter* been described from clinical specimens. They are widely distributed in the environment, especially in soil [3, 6]. Presently, the identification at species level requires special analysis (i.e., 16S rRNA gene sequence and peptidoglycan structure), because phenotypic characters are not sufficiently reliable to differentiate the 21 presently defined species [8–10]. To our knowledge, this is the first report of a case of subacute infective endocarditis due to *A. woluwensis* in an HIV-seronegative injection drug user.

Funke et al. [3] demonstrated that 4 of 11 clinical isolates of *Arthrobacter* species were representatives of 2 new species. The names of “*Arthrobacter cumminsi*” (to honor Cecil S. Cummins, one of the pioneers of chemotaxonomy) and “*A. woluwensis*” (from Woluwe, the town in Belgium where the first clinical isolate was recovered) were proposed.

The identification of coryneform bacteria represents an unusual challenge for the microbiology laboratory. In fact, microbiologists should not rely entirely on the database of commercial identification system, which only covers a restricted number of species. As in a previously reported case of *Arthropacter* bacteremia [11], our isolate was initially diagnosed as *C. aquaticum* (now called “*Leifsonia aquatica*”) [12]. The final identification of *A. woluwensis* required analysis by molecular genetic methods—that is, 16S rRNA PCR, direct sequencing, and a GenBank search. It has been speculated that clinical *Arthrobacter* strains may have been previously identified as CDC (Centers for Disease Control and Prevention) coryneform group B-1 and B-3 bacteria [13, 14].

In our patient, who, for many years, intravenously injected a broad range of illicit drugs, we were able to identify *A. woluwensis* as the etiologic agent of infective endocarditis of the native mitral valve. The potential for coryneform bacteria to cause endocarditis in injection drug users has been substantiated previously [15, 16].

*A. woluwensis* isolated from our patient was found, by the disk diffusion method, to be resistant to all penicillins and cephalosporins, a resistance pattern frequently observed in several coryneform bacteria. This is of significance, because, to date, the NCCLS has not recommended breakpoints for disk diffusion testing of coryneform bacteria. The susceptibility pattern determined using this method should be regarded as presumptive. However, the determination of MICs for the different antibiotics, as reported in Results, confirms the high intrinsic resistance of *A. woluwensis*, which is not seen in many other true *Arthrobacter* strains [3].

*Arthrobacter* species was reported as a cause of well-documented human disease only on 5 other occasions: 2 cases of bacteremia, 1 case of postoperative endophthalmitis, 1 case of Whipple disease–like syndrome, and 1 case of phlebitis (table
1). One explanation for the rarity of diseases caused by Arthrobacter species is the relatively low pathogenicity of this genus and, more generally, of coryneform bacteria. On the other hand, the precise identification of coryneform bacteria remains a challenge for most microbiology laboratories, and this could result in a bias toward a low recovery rate of Arthrobacter species. In fact, from our review of the literature, we were able to retrieve data about only 41 cases in which Arthrobacter species was isolated from a variety of human clinical samples (i.e., blood, urine, wound secretion, lymph node biopsy specimen, ocular fluid, fluid from external ear, vaginal fluid, and amniotic fluid) [3, 17–21]. Of these 41 reports, there are now 6 cases of clinical disease, including the case reported here. It seems likely that Arthrobacter species is part of the human skin flora. Moreover, particularly in the case of A. cumminsii, the isolation from urine, vaginal, and cervical samples indicates this microorganism is a possible resident of the genitourinary tract.

In conclusion, the identification of A. wolumensis as a cause of infective endocarditis in an injection drug user was only possible with the application of modern techniques of molecular diagnosis relying on PCR, direct DNA sequencing, and GenBank search. Attempts to identify the microorganism with standard biochemical characterization routinely performed in diagnostic laboratories produced misleading results, with the classification of it as C. aquaticum.

The identification of coryneform bacteria to the species level is important to detect unsuspected species, to ascribe potential pathogenicity to species so far thought to be nonpathogenic, and to outline hitherto undescribed species [22]. Moreover, this information should help the physician to distinguish among contamination, colonization, and infection, thus providing the patient with the best available antibiotic therapy.

Acknowledgment

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References