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Gluconobacter as Well as *Asaia* Species, Newly Emerging Opportunistic Human Pathogens among Acetic Acid Bacteria▽†

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Acetic acid bacteria (AAB) are broadly used in industrial food processing. Among them, members of the genera *Asaia*, *Acetobacter*, and *Granulibacter* were recently reported to be human opportunistic pathogens. We isolated AAB from clinical samples from three patients and describe here the clinical and bacteriological features of these cases. We report for the first time (i) the isolation of a *Gluconobacter* sp. from human clinical samples; (ii) the successive isolation of different AAB, i.e., an *Asaia* sp. and two unrelated *Gluconobacter* spp., from a cystic fibrosis patient; and (iii) persistent colonization of the respiratory tract by a *Gluconobacter* sp. in this patient. We reviewed the main clinical features associated with AAB isolation identified in the 10 documented reports currently available in the literature. Albeit rare, infections as well as colonization with AAB are increasingly reported in patients with underlying chronic diseases and/or indwelling devices. Clinicians as well as medical microbiologists should be aware of these unusual opportunistic pathogens, which are difficult to detect during standard medical microbiological investigations and which are multiresistant to antimicrobial agents. Molecular methods are required for identification of genera of AAB, but the results may remain inconclusive for identification to the species level.

Acetic acid bacteria (AAB) belong to the family *Acetobacteraceae* and oxidize alcohols or sugars, leading to the production of acetic acid. AAB, such as members of the genera *Acetobacter*, *Asaia*, *Gluconobacter*, and *Gluconacetobacter*, are commonly found in soil or are associated with plants (35). They have been used in industrial food processing throughout human history, especially to convert wine to vinegar and to produce tropical fermented products. The first report of infection with AAB in humans dates from 2004, when Snyder et al. reported a case of peritonitis associated with *Asaia bogorensis* in a patient with a peritoneal dialysis catheter (27). Greenberg et al. reported 2 years later that another AAB, *Granulibacter bethesdensis*, was the cause of infection in patients originating from geographically distinct locations and suffering from chronic granulomatous disease (CGD) (13, 14). Since then, AAB have increasingly been reported as organisms potentially infecting humans.

We isolated AAB from clinical samples from three patients consulting or hospitalized in two French tertiary-care teaching hospitals. In this paper, we describe the clinical and bacteriological features of these cases and present a summarized review of the 10 documented cases of AAB isolation previously reported in humans.

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Case 1. *Gluconobacter* sp. bacteremia in a nonimmunocompromised patient with a history of intravenous drug abuse. A 29-year-old HIV-negative man (patient 1), known to be an intravenous drug abuser, was hospitalized in October 2006 at the tertiary-care teaching hospital of Nancy, France, for a progressive decrease of visual acuity in his left eye. He was afebrile upon admission. Fungal endophthalmitis was suspected, and *Candida albicans* was isolated from a vitreous sample. A central venous catheter was placed for amphotericin B administration. Fever (39°C) appeared after 3 days with an increase in C-reactive protein level. No ultrasonographic signs of endocarditis were detected. Two peripheral blood samples were drawn. Both aerobic culture vials (Bactec Plus Aerobic/F medium; Becton Dickinson, Le Pont de Claix, France) yielded the growth of a Gram-negative rod (strain LBN 175) after 3 days of incubation at 35°C in a Bactec 9000 system (Becton Dickinson). The organism was subcultured on chromogenic CPS-ID2 medium (bioMérieux, Marcy l'Etoile, France). Tiny colonies appeared after 48 h of incubation at 37°C. Conventional methods and commercialized systems did not allow the identification of this catalase-positive, oxidase-negative rod, which was further identified to be a *Gluconobacter* sp. by mo-

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lecular means. The patient became afebrile after 24 h of antimicrobial treatment (ceftriaxone, 1 g/day for 10 days). The C-reactive protein level decreased progressively to normal values. To this day, no recurrence has been documented.

Case 2. Successive isolation of an *Asaia* sp. and of two unrelated *Gluconobacter* spp. in a CF patient. A 2-year-old boy (patient 2) suffering from cystic fibrosis (CF) consulted at the CF center of the tertiary-care teaching hospital of Montpellier, France, for routine evaluation in January 2008. CF was diagnosed at birth on the basis of positive sweat tests (chloride concentration $>$ 60 meq/liter) and an F508del homozygous genotype. He had a pancreatic insufficiency and presented with several episodes of rhinitis, but no antimicrobial therapy was recently given. Nutritional status and respiratory conditions were normal. Sputum analysis showed $<$25 polymorphonuclear leukocytes per microscopic field and yielded 8×10^2 CFU/ml of a catalase-positive, oxidase-negative, Gram-negative rod (strain aP75) growing in 3 days at 30°C on the *Burkholderia cepacia*-selective medium Cepacia agar (AES, Combourg, France) together with *Haemophilus influenzae* ($>10^7$ CFU/ml), *Moraxella catarrhalis* (8×10^4 CFU/ml), and a polymorphic commensal microflora. Two and 4 months later, two other isolates of atypical Gram-negative, oxidase-negative rods, strains aP78 and aP81, were recovered during routine analysis of sputum samples on Cepacia agar (3×10^3 and 10^3 CFU/ml, respectively). In the latter analysis, $>$25 polymorphonuclear leukocytes were observed per microscopic field, and the Gram-negative rod was the only notable isolate. In December 2009, after several sputum analyses, which did not reveal any atypical Gram-negative rod, a strain (strain aP112) presenting phenotypic characteristics similar to those of strains aP78 and aP81 was isolated from sputum at 6×10^2 CFU/ml. This strain was isolated together with $>10^6$ CFU/ml each of *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Moraxella catarrhalis*, and *Haemophilus influenzae*. The patient presented with increased cough, sputum production with a loss of appetite, and fatigue. On the basis of this respiratory exacerbation, a combination of amoxicillin-clavulanic acid and co-trimoxazole for 15 days was started, leading to clinical condition improvement. No significant lesions were observed on thoracic computed tomography (CT) scan. Phenotypic identification was unsuccessful for the four isolates, isolates aP75, aP78, aP81, and aP112, and molecular tools affiliated the first strain to *Asaia* sp. and the three others to *Gluconobacter* sp.

Case 3. *Gluconobacter* sp. in a second CF patient followed at the same CF center. A 3-year-old girl (patient 3) with CF was hospitalized at the tertiary-care teaching hospital of Montpellier for two successive fever episodes in August 2008. CF was diagnosed at birth on the basis of positive sweat tests and an F508del homozygous genotype. She had a pancreatic insufficiency, and her medical history included a central catheter infection by a methicillin-resistant coagulase-negative *Staphylococcus* 8 months earlier. At first examination, no inflammatory signs were apparent around the indwelling catheter. Blood cultures were negative. Sputum analysis showed $>$25 polymorphonuclear leukocytes per microscopic field and numerous Gram-negative rods. Cultures yielded 8×10^3 CFU/ml of a catalase-positive, oxidase-negative, Gram-negative rod (strain aP90) forming tiny, gray colonies in 2 days at 30°C on Cepacia agar together with *Candida parapsilosis*, *Serratia marcescens* (2×10^2 CFU/ml), and polymicrobial oral microflora. Phenotypic methods failed to identify the isolate, while molecular-based methods identified this strain as a *Gluconobacter* sp. Since the patient became afebrile spontaneously, no antimicrobial therapy was administered. Her clinical evolution was stable, with a normal nutritional status and no significant thoracic lesions on CT scan.

MATERIALS AND METHODS

Bacterial strains. Six strains, including one *Asaia* sp. isolate (aP75) and five *Gluconobacter* sp. isolates (LBN 175, aP78, aP81, aP90, and aP112), recovered from patients 1, 2, and 3 were included in the study and analyzed as described below.

Molecular-based identification. 16S rRNA gene amplification was performed as described previously (31). The housekeeping gene *dnaK* was amplified using primers dnaK-01-F and dnaK-02-R, as described by Cleenwerck et al. (4). 16S-23S rRNA gene internal transcribed spacer (ITS) region analysis was conducted as described by Yukphan et al. (36). PCR products were sequenced on an Applied Biosystems automatic sequencer (Beckman Coulter Genomics) by using a forward and/or a reverse primer. The sequences were compared with sequences deposited in databases using the standard nucleotide-nucleotide BLAST program (http://www.ncbi.nlm.nih.gov/BLAST/) and, for 16S rRNA genes sequences, using the sequence match system of the Ribosomal Database Project II database (http://rdp.cme.msu.edu). The sequences for similarity analysis were selected from the GenBank database using the BLAST program and taxonomy browser (http://www.ncbi.nlm.nih.gov). The sequences were aligned using CLC Sequence Viewer, version 5.11 (T. Knudsen et al., CLCbio). Unaligned nucleotides at both ends of the sequences were removed using sequence alignment editor software (Se-Al, version 2.0a11; http://tree.bio.ed.ac.uk/software/seal/). Levels of sequence identity were calculated from similarity tables constructed with aligned sequences in the DNADIST program using the PHYLIP, version 3.68, package (9).

Molecular typing. The five *Gluconobacter* sp. strains isolated from patients 1, 2, and 3 were submitted to pulsed-field gel electrophoresis (PFGE) in order to assess whether there was an epidemiological link between the strains. Genomic DNAs were prepared in agarose plugs and submitted to SpeI (New England Biolabs, Hertfordshire, United Kingdom) restriction, as described previously (31). SpeI fragments were separated by PFGE using a contour-clamped homogeneous electric field apparatus (CHEF-DRII; Bio-Rad, Hercules, CA) in a 1% agarose gel in 0.5× Tris-borate-EDTA buffer (TBE) at 10°C. Pulse ramps were 5 to 35 s for 28 h, followed by 2 to 10 s for 8 h at 4.5 V/cm. The PFGE patterns were visually compared and interpreted according to the criteria of Tenover et al. (30).

Phenotypic tests. All the isolates were tested for the ability to grow on the following agar media: *Burkholderia cepacia*-selective Cepacia agar medium (AES), CPS-ID2 agar, bromocresol purple (BCP) agar, MacConkey agar, Columbia blood sheep agar, chocolate-Polyvitex agar, and Mueller-Hinton (MH) agar (bioMérieux, Marcy l'Etoile, France). They were incubated for up to 5 days at 37°C or 30°C. Conventional tests were used for the detection of catalase and oxidase production. The following commercialized systems for identification of Gram-negative rods were used: API 20E strips, API 20NE strips, and GN cards on the Vitek2 system, version 03.01 (bioMérieux). All tests were performed as recommended by the manufacturer. Due to enhanced growth at 30°C (26), the API 20E strips were incubated at both 37°C and 30°C for 24 h. API biochemical profiles were converted to an identification by using APILAB Plus software, version 3.3.3 (bioMérieux). The main metabolic characteristics of AAB, i.e., acid tolerance, acetic acid production, and use of ethanol as the sole source of carbon, were tested using *Acetobacter* agar medium, a culture medium containing ethanol and calcium carbonate ($CaCO_3$) at pH 3.5 (2% glucose, 0.5% ethanol, 0.5% peptone, 0.8% yeast extract, 0.7% $CaCO_3$, 1.2% agar) and allowing the visualization of calcium carbonate clearing (34).

Antimicrobial susceptibility testing. Antimicrobial susceptibility testing was performed by using the AST-N052 card for aerobic Gram-negative bacilli (bioMérieux) on the Vitek2 system, according to the manufacturer's recommendations, and by the disk diffusion method on MH agar, according to the 2010 guidelines of the Comité de l'Antibiogramme de la Société Française de Microbiologie (http://www.sfm.asso.fr/publi/general.php?pa=1), except for incubation, which was performed at 30°C, since *Gluconobacter* spp. and *Asaia* spp. grow better at this temperature (1, 26). The following antibiotic disks were used: amoxicillin (AMX; 25 μg), amoxicillin-clavulanic acid (AMC; 20 μg/10 μg),

ticarcillin (TIC; 75 μg), ticarcillin-clavulanic acid (TIM; 75 μg/10 μg), piperacillin (PIP; 75 μg), piperacillin-tazobactam (TZP; 75 μg/10 μg), imipenem (IMP; 10 μg), ertapenem (ETP; 10 μg), meropenem (MEM; 10 μg), cephalothin (CEF; 30 μg), cefoxitin (FOX; 30 μg), cefpodoxime (CPD; 30 μg), cefotaxime (CTX; 30 μg), ceftriaxone (CRO; 30 μg), ceftazidime (CAZ; 30 μg), cefpirome (CPO; 30 μg), cefepime (FEP; 30 μg), moxalactam (MOX; 30 μg), aztreonam (ATM; 30 μg), gentamicin (GEN; 10 IU), tobramycin (TOB; 10 μg), netilmicin (NET; 30 μg), amikacin (AMK; 30 μg), isepamicin (ISP; 30 μg), chloramphenicol (CHL; 30 μg), tetracycline (TET; 30 μg), doxycycline (DOX; 30 μg), tigecycline (TIG; 15 μg), colistin (CS; 50 μg), trimethoprim-sulfamethoxazole (SXT; 1.25 μg/23.75 μg), nalidixic acid (NAL; 30 μg), ofloxacin (OFX; 5 μg), ciprofloxacin (CIP; 5 μg), levofloxacin (LVX; 5 μg), and fosfomycin (FOF; 50 μg). Except for the ceftriaxone, doxycycline, tigecycline, ertapenem, and meropenem disks, which were purchased from Oxoid (Hampshire, England), the disks were purchased from Bio-Rad (Marnes-la-Coquette, France). MICs of amoxicillin-clavulanic acid, imipenem, meropenem, ertapenem, doripenem, ceftazidime, ceftriaxone, tetracycline, and tigecycline were determined using Etest strips (AB Biodisk, Solna, Sweden), according to the recommendations of the manufacturer, except for incubation, which was performed at 30°C. Considering the absence of interpretative breakpoints for susceptibility testing of AAB, the results were comparatively analyzed.

Nucleotide sequence accession numbers. The GenBank accession numbers for the 16S rRNA gene, 16S-23S rRNA gene ITS, and *dnaK* gene sequences determined in this work for the six AAB clinical isolates are indicated in Table 1.

RESULTS

Molecular-based identification. Partial to nearly complete (880- to 1,409-bp) 16S rRNA gene sequences were obtained for the six isolates. On the basis of the 16S rRNA gene sequences identities of >99% with closest relatives, strain aP75 belonged to the genus *Asaia* and the five other isolates belonged to the genus *Gluconobacter*. These five *Gluconobacter* sp. strains shared more than 99.6% of their nucleotide positions. However, the isolates could not be identified to the species level due to a low 16S rRNA gene polymorphism in the *Asaia* and *Gluconobacter* genera (Table 1). With the aim to resolve the strains' identification, we performed 16S-23S rRNA gene ITS sequencing, previously shown to be a valuable tool for species identification in several genera of AAB (29). The *Asaia* sp. isolate was most closely related to *Asaia krungthepensis* and *A. bogorensis* type strains, but with no more than 98.2 to 98.3% ITS sequence similarity (Table 1). Moreover, comparative analysis with the *Asaia spathodeae* sequence could not be performed because the latter was not available in the databases, thereby preventing any identification to the species level. The five *Gluconobacter* sp. isolates displayed 98 to 100% 16S-23S rRNA gene ITS sequence similarity with each other and were most closely related to *Gluconobacter japonicus* (Table 1). However, they formed a heterogeneous group of strains. Strains aP78 and aP81 showed a high level of sequence identity (99.7%) with the *G. japonicus* type strain and a distance score of 1.8% to the next-closest species, *Gluconobacter frateurii* (Table 1). These data were in agreement with both the intraspecies and interspecies variability described by Malimas et al. (21) for *G. japonicus*, but further tests are still required to determine if strains aP78 and aP81 represent the first human isolates of *G. japonicus*. Indeed, a similarity table drawn with 18 *G. frateurii* and 9 *G. japonicus* 16S-23 rRNA gene ITS region sequences showed (i) a *G. japonicus* intraspecies variability ranging from 98.2 to 100%, (ii) a *G. frateurii* intraspecies variability ranging from 97 to 100%, and (iii) an interspecies *G. japonicus*/*G. frateurii* variability that may surpass intraspecies variability (data not shown). In contrast, the three other *Gluconobacter* strains displayed no more than 98.2 to 98.9% sequence similarity with the *G. japonicus* type strain, and a lower level of discrimination was observed with *G. frateurii.* Thus, these strains could not be unambiguously affiliated with a *Gluconobacter* species. It is noteworthy that interpretation of the relationship of the sequenced strains was hindered due to incorrect labeling of several *G. japonicus* ITS sequences in the database (*G. japonicus* strains NBRC 3260, NBRC 3263, NBRC 3269, NRBC 3271^T, and NBRC 3272 appearing under the *G. frateurii* nomenclature with GenBank accession numbers AB163836, AB206585, AB206587, AB162709 and AB163847 respectively, and strains NBRC 3263 and NBRC 3269 also being deposited as *Gluconobacter cerinus* strains with GenBank accession numbers AB163838 and AB163844, respectively). Three housekeeping genes (*dnaK*, *groEL*, and *rpoB*) were recently proposed for use for differentiation of AAB species (4). Among them, we used *dnaK* gene sequences and showed that the *Gluconobacter* isolates shared more than 97.7% of their sequences and that they displayed 90 to 95.9% identity with sequences available for *Gluconobacter* species, with *G. cerinus* and *Gluconobacter thailandicus* being the two most closely related species. The *Asaia* strain shared 98.1% of its *dnaK* sequence with that of the type strain of the closest species, *A. bogorensis* (Table 1). These data did not contribute to the identification to the species level of the *Asaia* and *Gluconobacter* strains due to low levels of sequence similarity with known species and to the absence of deposited sequences for *G. japonicus*, *Asaia lannaensis*, and *A. spathodeae*.

Molecular fingerprinting. PFGE-based typing (Fig. 1) showed that patient 2 was colonized over a 2-month period with the same *Gluconobacter* sp. strain, since strain aP78 was indistinguishable from strain aP81, and 19 months later experienced a second episode of colonization involving an unrelated strain (aP112). No clonal relatedness could be demonstrated between *Gluconobacter* sp. strains isolated from sputum samples from patients 2 and 3, thereby excluding the possibility of cross-contamination between the two patients consulting at the same CF center. The *Gluconobacter* sp. strain isolated at the tertiary-care teaching hospital of Nancy also displayed a distinct PFGE pattern. These observations highlight the existence of genomic diversity among the *Gluconobacter* clinical isolates.

Phenotypic features. The six AAB strains tested grew in 24 to 48 h at 30°C on Cepacia agar, CPS-ID2 agar, BCP agar, Columbia blood sheep agar, chocolate-Polyvitex agar, and MH agar, whereas culture on the same media at 37°C yielded very tiny colonies. Growth was not observed on MacConkey agar at 37°C or 30°C. Strains were oxidase negative and catalase positive. Colonies obtained from all isolates cleared *Acetobacter* agar medium, thereby confirming the acetic acid production by all the strains and their affiliation to the AAB group. Other phenotypic characteristics are presented in Table 1. The same biochemical profiles were observed on API 20E strips incubated at 30°C or at 37°C for all the clinical isolates. The genomically unrelated *Gluconobacter* sp. strains displayed identical biochemical characteristics with each of the three commercialized systems tested. With these methods, strains either were not identified or were misidentified (*Gluconobacter* sp. strains identified as either *Acinetobacter* spp. or *Shigella*

TABLE 1. Characteristics of the six AAB strains isolated from three patients

Characteristic	Patient 1 (strain LBN 175)	Patient 2				Patient 3 (strain aP90)
		Strain aP75	Strain aP78	Strain aP81	Strain aP112	
Isolation data						
Sample	Blood	Sputum	Sputum	Sputum	Sputum	Sputum
Date	October 2006	January 2008	March 2008	May 2008	December 2009	August 2008
Genotypic characteristics						
16S rRNA gene						
Sequence GenBank accession no.	HM051360	HM051372	HM051358	HM051359	HM051356	HM051357
Type strains of most closely related species (% identity)	*G. frateurii*^{T} (99.9), *G. japonicus*^{T} (99.9), *G. thailandicus*^{T} (99.6), *G. cerinus*^{T} (99.4), *G. kondonii*^{T} (99.1)	*A. siamensis*^{T} (99.8), *A. bogorensis*^{T} (99.7), *A. spathodeae*^{T} (99.7), *A. krungthepensis*^{T} (99.4), *A. lannaensis*^{T} (99.2)	*G. frateurii*^{T} (100), *G. japonicus*^{T} (100), *G. thailandicus*^{T} (99.7), *G. cerinus*^{T} (99.5), *G. kondonii*^{T} (99.2)	*G. frateurii*^{T} (100), *G. japonicus*^{T} (100), *G. thailandicus*^{T} (99.7), *G. cerinus*^{T} (99.5), *G. kondonii*^{T} (99.2)	*G. frateurii*^{T} (99.7), *G. japonicus*^{T} (99.7), *G. thailandicus*^{T} (99.4), *G. cerinus*^{T} (99.2), *G. kondonii*^{T} (98.9)	*G. frateurii*^{T} (100), *G. japonicus*^{T} (100), *G. thailandicus*^{T} (99.7), *G. cerinus*^{T} (99.5), *G. kondonii*^{T} (99.2)
16S-23S rRNA gene ITS						
Sequence GenBank accession no.	HM051361	HM051373	HM051362	HM051363	HM051364	HM051365
Type strains of most closely related species (% identity)	*G. japonicus*^{T} (98.9), *G. frateurii*^{T} (97.2), *G. thailandicus*^{T} (96.6), *G. cerinus*^{T} (95.4)	*A. krungthepensis*^{T} (98.3), *A. bogorensis*^{T} (98.2), *A. siamensis*^{T} (96.8), *A. lannaensis*^{T} (96.5)	*G. japonicus*^{T} (99.7), *G. frateurii*^{T} (97.9), *G. thailandicus*^{T} (97.2), *G. cerinus*^{T} (95.8)	*G. japonicus*^{T} (99.7), *G. frateurii*^{T} (97.9), *G. thailandicus*^{T} (97.2), *G. cerinus*^{T} (95.8)	*G. japonicus*^{T} (98.7), *G. frateurii*^{T} (97.9), *G. thailandicus*^{T} (97.6), *G. cerinus*^{T} (96.4)	*G. japonicus*^{T} (98.2), *G. frateurii*^{T} (97.7), *G. thailandicus*^{T} (97.2), *G. cerinus*^{T} (96.4)
dnaK gene						
Sequence GenBank accession no.	HM051366	HM051371	HM051367	HM051368	HM051369	HM051370
Type strains of most closely related species (% identity)	*G. thailandicus*^{T} (95.7), *G. cerinus*^{T} (95.4), *G. frateurii*^{T} (94.8)	*A. bogorensis*^{T} (97.6), *A. krungthepensis*^{T} (94.3), *A. siamensis*^{T} (92.9)	*G. thailandicus*^{T} (95.5), *G. cerinus*^{T} (95.5), *G. frateurii*^{T} (94.6)	*G. thailandicus*^{T} (95.5), *G. cerinus*^{T} (95.5), *G. frateurii*^{T} (94.6)	*G. cerinus*^{T} (95.9), *G. thailandicus*^{T} (95.5), *G. frateurii*^{T} (94.6)	*G. thailandicus*^{T} (95.2), *G. cerinus*^{T} (95.2), *G. frateurii*^{T} (94.2)
Molecular fingerprinting						
PFGE pattern	A	ND[e] ND	B	B	C	D
Phenotypic characteristics[a]						
API 20E strip (24 h, 37°C)						
Numerical profile	0004042	0000000	0004042	0004042	0004042	
Positive reactions[b]	GLU, MEL, ARA	None	GLU, MEL, ARA	GLU, MEL, ARA	GLU, MEL, ARA	
Taxon (% identity)	*Acinetobacter baumannii*/*A. calcoaceticus* (95), *Shigella* group (5)	NA[f]	*Acinetobacter baumannii*/*A. calcoaceticus* (95), *Shigella* group (5)	*Acinetobacter baumannii*/*A. calcoaceticus* (95), *Shigella* group (5)	*Acinetobacter baumannii*/*A. calcoaceticus* (95), *Shigella* group (5)	
Identification rating	Low discrimination	No identification	Low discrimination	Low discrimination	Low discrimination	
API 20NE strip (48 h, 30°C)						
Numerical profile	4044000	0465400	4044000		4044000	
Positive reactions[c]	GLU, GLUa, MANa	ESC, PNPG, GLUa, ARAa, MANa, GNTa	GLU, GLUa, MANa		GLU, GLUa, MANa	
Taxon (% identity)	*Aeromonas salmonicida masoucida*/*A. achromogenes*, *Pasteurella* spp., *Acinetobacter lwoffii*	*Sphingomonas paucimobilis* (97.6)	*Aeromonas salmonicida masoucida*/*A. achromogenes*, *Pasteurella* spp., *Acinetobacter lwoffii*		*Aeromonas salmonicida masoucida*/*A. achromogenes*, *Pasteurella* spp., *Acinetobacter lwoffii*	
Identification rating	Unacceptable	Good identification	Unacceptable		Unacceptable	

Vitek2 GN card					
Numerical profile	2105410400001210	2005510520001210	2105410400001210	2105410400001210	2105410400001210
Positive reactions[d]	GlyA, O129R, ADO, dMAN, lARL, dGLU, dMNE, CMT, OFF, dSOR	BGLU, GlyA, O129R, ADO, dTAG, dMAN, dGLU, dMNE, TyrA, CMT, OFF, dSOR	GlyA, O129R, ADO, dMAN, lARL, dGLU, dMNE, CMT, OFF, dSOR	GlyA, O129R, ADO, dM AN, lARL, dGLU, dMNE, CMT, OFF, dSOR	GlyA, O129R, ADO, dMAN, lARL, dGLU, dMNE, CMT, OFF, dSOR
Taxon	*Shigella* group	Unidentified organism	*Shigella* group	*Shigella* group	*Shigella* group
Identification rating	Good	NA	Good	Good	Good
Antimicrobial suspectibility pattern[a]					
Diam (mm) by disk diffusion assay					
AMC	12	6	16	18	12
TIM	16	6	16	17	16
TZP	15	6	14	16	14
IMP	38	13	30	28	32
MEM	34	6	34	33	26
CRO	14	14	17	21	12
CAZ	38	6	36	25	31
GEN	38	18	27	22	23
TOB	36	13	22	19	25
NET	36	22	27	22	22
AMK	28	11	18	16	21
ISP	30	11	18	16	21
TET	38	22	32	28	36
DOX	34	31	31	32	33
TIG	27	18	26	24	28
FOF	26	11	25	24	17
Etest MIC (mg/liter)					
Amoxicillin-clavulanic acid	48/2	ND	24/2	16/2	48/2
Imipenem	4	ND	4	3	3
Meropenem	2	ND	3	1.5	4
Doripenem	4	ND	4	2	4
Ertapenem	>32	ND	>32	>32	>32
Ceftriaxone	48	ND	48	16	12
Ceftazidime	4	ND	4	1	1.5
Tetracycline	0.38	0.5	0.75	0.5	0.38
Tigecycline	1.5	2	1.5	1	1.5

[a] Data for genotypically unrelated isolates; clonal strains aP78 and aP81 shared an identical phenotype.
[b] Positive reactions on API 20E strips: ARA, arabinose; GLU, glucose; MEL, melibiose.
[c] Positive reactions on API 20NE strips: ARAa, arabinose assimilation; ESC, esculin hydrolysis; GLU, glucose fermentation; GLUa, glucose assimilation; GNTa, gluconate assimilation; MANa, mannitol assimilation; PNPG, para-nitrophenyl-beta-D-galactopyranoside.
[d] Positive reactions on Vitek2 GN card: ADO, adonitol; BGLU, beta-glucosidase; CMT, coumarate; dGLU, D-glucose; dMAN, D-mannitol; dMNE, D-mannose; dSOR, D-sorbitol; dTAG, D-tagatose; GlyA, glycine arylamidase; lARL, L-arabitol; O129R, resistance to compound O129; OFF, glucose fermentation; TyrA, tyrosine arylamidase.
[e] ND, not determined.
[f] NA, not applicable.

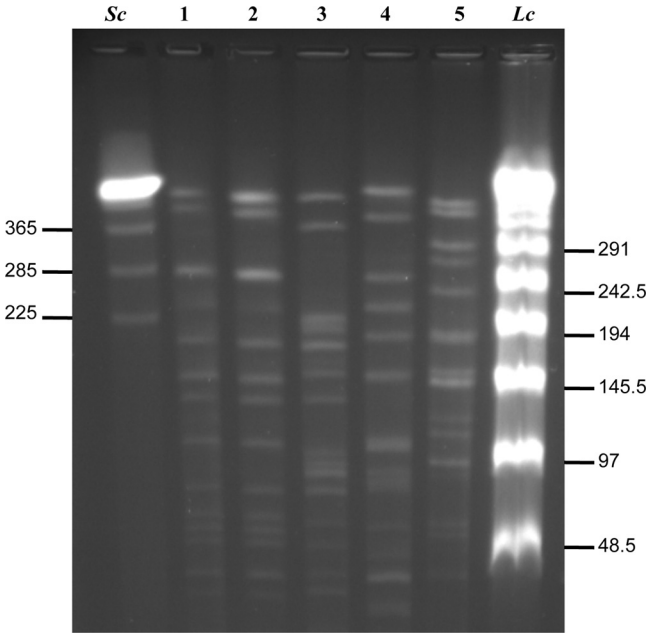

FIG. 1. PFGE patterns of SpeI-restricted DNAs for the five *Gluconobacter* sp. strains reported in this study. Lanes 1, 2, and 3, strains aP78, aP81, and aP112 (case 2), respectively; lane 4, strain aP90 (case 3); lane 5, strain LBN 175 (case 1); lanes *Sc* and *Lc*, *Saccharomyces cerevisiae* and concatemer of phage lambda DNAs (Bio-Rad), respectively, used as molecular size markers. Sizes are indicated in kilobases.

spp. [low discrimination] by using the API 20E system; *Gluconobacter* sp. strains identified as *Shigella* spp. [good identification] by using the Vitek2 GN system; the *Asaia* sp. strain identified as *Sphingomonas paucimobilis* [good identification] by using the API 20NE system).

Antimicrobial susceptibility patterns. No results were obtained using the Vitek2 Gram-negative panel due to insufficient growth. Using the disk diffusion method, no inhibition zone or narrow inhibition zones (≤14 mm) were observed for almost all antibiotics (AMX, TIC, PIP, ETP, CEF, FOX, CTX, CPD, CPO, FEP, MOX, ATM, CHL, CS, SXT, NAL, OFX, CIP, LVX) for all the clinical isolates tested. Antimicrobial agents for which inhibition diameters of >14 mm were observed for the *Gluconobacter* or *Asaia* isolates are reported in Table 1. On the basis of these data, ceftazidime, imipenem, meropenem, aminoglycosides (particularly gentamicin, tobramycin, and netilmicin), tetracycline, doxycycline, tigecycline, and fosfomycin were the agents most active against the *Gluconobacter* isolates. The strains exhibited high Etest MIC values for amoxicillin-clavulanic acid, ceftriaxone, and ertapenem, while the MICs were lower for the other carbapenems tested, as well as for ceftazidime, tetracycline, and tigecycline (Table 1). Against the *Asaia* clinical isolate, doxycycline, tetracycline, netilmicin, and gentamicin were the most active drugs.

Comparative clinical characteristics of patients. Clinical data for our patients together with those of the 10 documented cases previously reported in the literature are summarized in the table in the supplemental material. The two cases of AAB isolation in CF patients reported herein are the first cases of human colonization with this group of bacteria. The pathogenicity of the *Gluconobacter* strain isolated from patient 1, although suspected, could not be definitely proven, as is the case for most of the other cases reported in the literature. Of interest is that underlying chronic diseases, a history of illegal drug abuse, and/or the presence of indwelling devices were reported for all patients.

DISCUSSION

Until 2006, *A. bogorensis* was the only AAB reported to be causing human disease (27). In 2006, Greenberg et al. reported a case of recurrent idiopathic lymphadenitis due to *G. bethesdensis* in a patient with CGD and demonstrated that Koch's postulates were fulfilled (14). Since then, several reports have described the isolation of *A. bogorensis* and *G. bethesdensis* from humans and suggested that these bacteria emerged as human pathogenic bacteria, as reviewed in the table in the supplemental material (10, 15, 20). Other isolates belonging to additional AAB genera and species, like *Acetobacter indonesiensis*, *Acetobacter cibinongensis*, and the recently described *A. lannaensis*, have been recovered from human clinical samples during bacteremia or in the course of CF, thereby confirming this emergence (see the table in the supplemental material) (1, 3, 12, 17). As illustrated by the three cases documented here, *Gluconobacter* sp. should be considered an additional AAB able to colonize or infect humans. In our report, AAB, including one *Asaia* sp. and four *Gluconobacter* sp. isolates, have been recovered from the respiratory tract of two CF patients, which is about 1% of the patients attending the CF center of Centre Hospitalier Universitaire de Montpellier. In these cases, the AAB were not incriminated in the evolution of the disease because of a favorable clinical evolution without any specific treatment. The only previous case of AAB isolation during CF has been reported from a respiratory tract infection caused by *A. indonesiensis* (3). Our report reinforces the hypothesis that AAB may specifically colonize and potentially infect the respiratory tract of CF patients by their propensity to grow in an acidic environment, which is a classic condition in the CF airway liquid (22). AAB were also implied or suspected to be responsible for other types of infections, including, in particular, bacteremia in patients for whom the probable source of infection was an indwelling device (1, 12, 17, 27). The known use of acidic substances such as vinegar or lemon juice to dilute heroin (2, 28) and the potential contamination of batches of compounds for intravenous drug abuse by AAB (32, 33) make us speculate that patient 1 was contaminated after intravenous drug injection, probably during his hospitalization, as he was apyretic at the time of admission. An improvement of the clinical condition of this immunocompetent patient was observed with ceftriaxone treatment, even though this molecule was not active *in vitro*, without removing the central venous catheter placed for antifungal drug administration. A similar observation has been reported during a case of bacteremia involving *A. bogorensis* in an immunocompetent patient whose clinical condition improved before the beginning of the treatment (32). AAB infections seemed to be more severe in immunocompromised patients, requiring removal of the suspected indwelling device and/or administration of an adapted antibiotic therapy for improvement of the clinical condition (1, 3, 17, 20, 27).

Asaia and *Gluconobacter* species are ubiquitous environmental AAB; flowers and fruits in tropical and temperate countries represent their major recognized natural habitat (19, 34, 35). They were also recovered from plant-derived natural products and from diverse sugary niches (16, 25). Besides, the genus *Gluconobacter* is of great industrial interest because of its use in vinegar and fermented food production and of its numerous biotechnological applications (6, 7, 8, 23). Little is known about the transmission of these emerging AAB pathogens to humans, but food may represent a source for AAB, as suggested previously for bears (11). Cases of contamination with strains thought to be exotic in patients living in temperate countries who have no history of travel in tropical countries or exotic food consumption are more and more frequently reported, suggesting that these species may not be restricted to particular geographic areas or natural niches (see the table in the supplemental material) (3, 12, 28, 32). For our patients, the route of contamination was not obvious except for patient 1, for whom a contamination occurring during drug injection was suspected. Juretschko et al. recently reported two cases of *A. lannaensis* bacteremia, which were suspected to be nosocomially related (17). In our study, both patients colonized with a *Gluconobacter* sp. received care in the same CF center, but cross-contamination was ruled out using the PFGE assay proposed in this study, which revealed genomic diversity of the strains and which supported the potential diversity of contamination sources.

None of the current commercialized systems for bacterial identification are able to recognize AAB, and *Gluconobacter* sp. could even be misidentified as a *Shigella* sp. by the Vitek2 system, probably due to the test conditions, i.e., incubation at 37°C for less than 12 h. However, the cultural characteristics of the strains, particularly the absence of growth on MacConkey medium, avoided any confusion. Molecular methods for identification are therefore required. Various microorganisms previously described to be merely environmental or plant or animal pathogens, such as *Caulobacter* spp., *Schineria* spp., or some AAB, were identified from human clinical samples by 16S rRNA gene sequencing (18, 24, 27). However, some AAB species may not be discriminated by 16S rRNA gene sequencing. In all previous case reports involving *Asaia* spp., the final identification at the species level was achieved using partial 16S rRNA gene sequencing. Among them, *A. bogorensis* was identified on the basis of more than 99% 16S rRNA gene sequence identity with *A. bogorensis* strains in two cases (27, 32), but this identification remains questionable because the type strains of the five currently described *Asaia* species shared more than 99.5% of their 16S rRNA gene positions and because the species related to *A. bogorensis*, i.e., *A. lannaensis* and *A. spathodeae*, were not described at the time of the case reports. The discrimination between some closely related *Gluconobacter* species may also remain unsuccessful. In these cases, 16S rRNA gene sequencing should therefore be restricted to use for genus-level identification. Other molecular techniques, such as restriction analysis of 16S-23S rRNA gene ITS regions (36) and, more recently, amplified fragment length polymorphism DNA fingerprinting (5) or sequencing of housekeeping gene *dnaK*, *rpoB*, or *groEL* (4), have been used for species differentiation in several genera of the family *Acetobacteraceae*. However, the 16S-23S rRNA gene ITS regions and *dnaK* gene sequencing used did not allow accurate species-level identification of the *Asaia* or the *Gluconobacter* isolates due to a lack of discriminatory power and/or incomplete databases. These molecular tools would probably be helpful in the precise recognition of some AAB species involved in human infection or colonization when the databases become completed with sequences from all species, particularly species in the genera *Gluconobacter* and *Asaia*, and when inaccurate species names are amended; but new molecular tools allowing a more accurate discrimination between some species might also be needed.

The most important problem raised by the isolation of AAB from human infections is the choice of antimicrobial treatment, especially since multiresistance is a common trait in most AAB isolated from human clinical samples (1, 3, 17, 27, 33). We found that the *Asaia* isolate presented a susceptibility pattern similar to the patterns reported previously (1, 17, 33), with aminoglycosides and cyclines being the only drugs active *in vitro*. The *Gluconobacter* isolates shared similar susceptibility profiles, with cyclines, aminoglycosides, carbapenems, and fosfomycin being the molecules exhibiting the highest levels of inhibitory activity *in vitro*. Despite the lack of established interpretative criteria for AAB antimicrobial susceptibility testing, we recommend the use of diffusion susceptibility testing methods, particularly MIC determination using Etest strips, when available, to support clinicians in their choice of antibiotic treatment.

Infrequently cultured from human samples, AAB are increasingly recognized as emerging human opportunistic pathogens. Their frequency and diversity may probably be underestimated because of their growth characteristics, particularly their faint growth at 37°C, a default temperature setting in routine medical microbiology, and because of the difficulty with identifying these microorganisms. We confirmed herein that underlying conditions are constantly associated with AAB isolation and, in particular, documented the second and third cases of AAB isolation from CF patients. We report for the first time that multiresistant strains belonging to the genus *Gluconobacter* may colonize or infect humans, highlighting the fact that this microorganism should be considered a new opportunistic human pathogen.

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