Gallibacterium anatis Bacteremia in a Human

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We describe the first case of bacteremia due to *Gallibacterium anatis*. The patient, a 26-year-old woman, developed bacteremia and diarrhea. The origin of infection was possibly due to a diet contaminated by *G. anatis* in this highly immunocompromised patient.

CASE REPORT

On 24 January 2013, a 26-year-old woman was admitted to the nephrology ward at Nantes University Hospital, in order to have monitored her chronic kidney insufficiency (renal biopsy) due to cystic fibrosis disease. In May 2011, she received a bipulmonary transplant. The immunosuppressive treatment included mycophenolate mofetil (2 g per day), tacrolimus (10 mg per day), and corticoids (9 mg per day). During hospitalization, she suffered abdominal pain, fever, and recent diarrhea. A complete blood cell count showed bicytopenia with neutropenia (0.93 × 10^9 cells/liter) and anemia (hemoglobin, 80 g/liter). Her C-reactive protein level was 46.4 mg/liter (normal range, <5 mg/liter). An empirical antimicrobial treatment was started with imipenem-cilastatin (1.5 g per day) and tobramycin (650 mg every 72 h, adapted to chronic kidney insufficiency). Four aerobic and anaerobic blood cultures (Bactec FX; Becton, Dickinson, Sparks, MD) were performed on the peripheral site over the course of 48 h. Concomitantly, a primary cytomegalovirus (CMV) infection with gastrointestinal, liver, and lung involvement was diagnosed. The viral investigation revealed a high cytomegalovirus viremia by real-time PCR (viral load, 501,971 copies/ml). Despite the broad-spectrum antimicrobial therapy, the first aerobic blood culture yielded Gram-negative bacilli (laboratory reference no. NTS31300851) after 17 h of incubation. Other blood cultures remained negative after 5 days of incubation. This bacterium was not hemolytic on blood agar (bioMérieux, Marcy l'Etoile, France), with negative oxidase and indole tests. The semiautomated api20E gallery (bioMérieux, Marcy l'Etoile, France) provided low discrimination identification with different taxons (*Escherichia coli* 2, *Shigella* species, *Pantoea* sp. 1, *Pasteurella pneumotropica*/*Mannheimia haemolytica*, which are species in the bioMérieux database associated with the semiautomated api20E), whereas the ID-GN card of the Vitek2 system (bioMérieux) failed to identify this bacterium (*E. coli*, *Shigella* species, *Pantoea* species). The 16S rRNA gene amplification and sequencing were performed with universal primers 27f and 1378r as previously described (1). The 1,360-bp fragment obtained matched that of *G. anatis*, with 99% similarity according to BIBI database (http://umr5558-sud-str1.univ-lyon1.fr/lebibi/lebibi.cgi) or BLAST (http://www.ncbi.nlm.nih.gov/BLAST) analysis. To confirm the identification of this unusual bacterium in human clinical specimens, *sodA* amplification and sequencing were also performed as previously described (2). The 451-bp fragment obtained matched that of *G. anatis*, with 99% similarity according to BIBI database (http://umr5558-sud-str1.univ-lyon1.fr/lebibi/lebibi.cgi) or BLAST

(http://www.ncbi.nlm.nih.gov/BLAST) analysis. Phylogenetic analysis with either the neighbor-joining or maximum-parsimony algorithm matched the NTS31300851 strain to the genus *Gallibacterium* and the species *G. anatis* by using MEGA5 software with tree constructions (Fig. 1a and b). The strain reduced nitrate to nitrite, produced α-glucosidase, and presented positive glucose, mannitol, and saccharose assimilation with api20E and ID-GN biochemical galleries. Unlike *E. coli*, the strain was negative for lysine decarboxylase, ornithine decarboxylase, and indole. Also, negative reactions for sorbitol, rhamnose, amygdaline, and arabinose assimilation differentiated the strain from *Pantoea* sp. 2.

Concerning *Gallibacterium* species, no European Committee on Antimicrobial Susceptibility Testing (EUCAST) specific recommendation for susceptibility testing exists. Therefore, *in vitro* susceptibility testing was performed using the Vitek2 system, and results were interpreted according to EUCAST guidelines. The bacterium was fully susceptible, except to fluoroquinolones (ofloxacin MIC ≥ 8 mg · liter^{-1}; ciprofloxacin MIC ≥ 4 mg · liter^{-1}), and cotrimoxazole (MIC ≥ 320 mg · liter^{-1}). Therefore, antimicrobial drug treatment was changed, on January 27, to ceftriaxone (1 g per day) and tobramycin (650 mg every 72 h).

On January 30, the patient was admitted to the pneumology ward but developed hypoxemia. She was transferred on February 4 to the intensive care unit due to bacteremia, hypoxemia, CMV sepsis, and metabolic acidosis. The patient died on March 3 due to pulmonary fibrosis, CMV sepsis, and anuric renal failure.

The genus *Gallibacterium*, first described by Christensen et al. in 2003, belongs to the family *Pasteurellaceae*, which includes diverse bacteria, including *Haemophilus*, *Aggregatibacter*, *Actinobacillus*, *Pasteurella*, *Mannheimia*, *Lonepinella*, and *Phocoenobacter*, classified into different genera based on metabolic properties (3, 4). *Gallibacterium* contains six different species that demonstrate similarities in 16S rRNA gene sequences and in phenotypic and polyamine contents: *Gallibacterium melopsittaci*, *Gallibacterium*
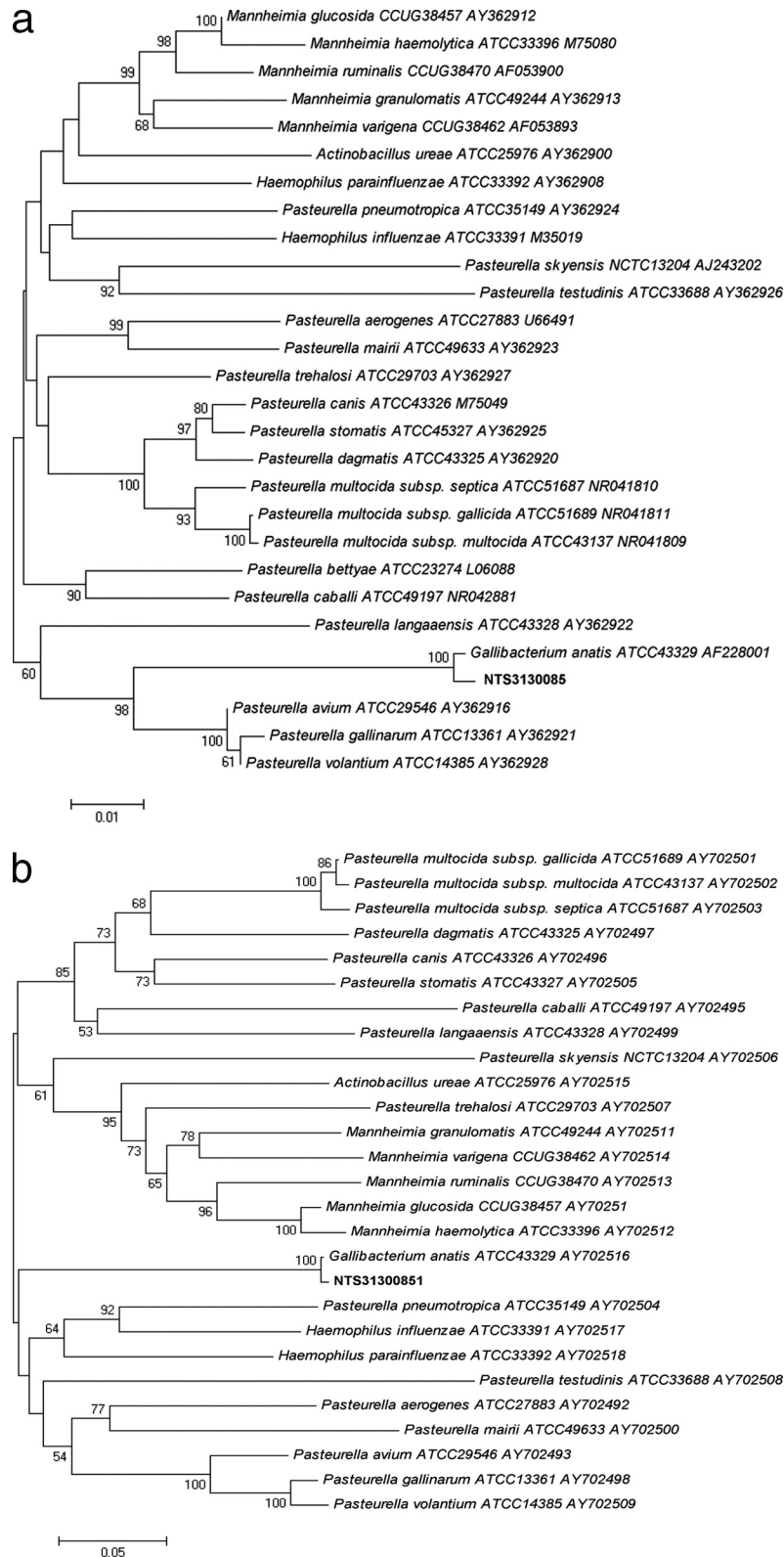
FIG 1 (a and b) Neighbor-joining (NJ) tree showing the phylogenetic placement of strain NTS31300851 (in boldface) among members of the *Gallibacterium anatis* species. Twenty-seven 16S rRNA gene and *sodA* sequences selected from the GenBank database were aligned with that of strain NTS31300851 by using MEGA5 (www.megasoftware.net). Accession numbers are indicated after the species name. The evolutionary history was inferred using the NJ method. The figure shows the optimal tree; the sums of the branch lengths for 16S rRNA genes and *sodA* genes were 0.61902303 and 2.62153347, respectively. The percentages of replicate trees in which the associated taxa clustered together in the bootstrap test (1,000 replicates) are shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Kimura 2-parameter method and are in the units of the number of base substitutions per site. The final data set contains 1,360 and 451 positions, for 16S rRNA genes and *sodA* genes, respectively. Phylogenetic analyses were conducted in MEGA5. NJ and parsimony trees were globally congruent with the distance tree and confirmed the placement of the NTS31300851 strain in the *Gallibacterium anatis* species. Scale bar indicates substitutions per nucleotide position. (a) 16S rRNA gene NJ tree showing the phylogenetic placement of strain NTS31300851 (in boldface); (b) *sodA* NJ tree showing the phylogenetic placement of strain NTS31300851 (in boldface).

trehalosifermentans, *Gallibacterium* genospecies III, *Gallibacterium salpingitidis*, *Gallibacterium* group V, and *Gallibacterium anatis* (5–7). *Gallibacterium anatis* has been isolated from various birds or other animals, such as chickens, ducks, geese, guinea fowl, turkeys, psittacine birds, partridges, cattle, budgerigars, and pigs (8). *G. anatis* was found to be prevalent in the upper respiratory tracts and the lower genital tracts of healthy chickens (5). However, isolates have also been recovered from a range of pathological lesions in chickens with bacteremia, oophoritis, follicle degeneration, salpingitis, peritonitis, enteritis, and respiratory tract diseases (5). The ATCC 43329 type strain was recovered from the intestinal track of a duck (3). The genus consists of Gram-negative, nonmotile, rod-shaped, or pleomorphic bacteria. The bacteria are catalase, oxidase, and phosphatase positive. Nitrate is reduced and acid is produced without gas formation from glycerol, $(-)$D-ribose, $(+)$D-xylose, $(-)$D-mannitol, $(-)$D-fructose, $(+)$D-galactose, $(+)$D-glucose, $(+)$D-mannose, sucrose, and raffinose. The *Gallibacterium* genus can be separated from other genera of *Pasteurellaceae* by differences in catalase, in symbiotic growth, in hemolysis, in urease, in indole, in acid production from $(+)$D-xylose, $(-)$D-mannitol, $(-)$D-sorbitol, and $(+)$D-mannose, in maltose, in raffinose, in dextrin, and in *o*-nitrophenyl-β-D-galactopyranoside (ONPG) and *p*-nitrophenyl-β-D-glucoside (PNPG) tests (3).

We describe in this report a case of human bacteremia due to *G. anatis*. This veterinary pathogen behaving as an opportunistic pathogen was able to cause an infection in a severely immunosuppressed woman. The source of bacteremia was not clearly established, but the most likely hypothesis is the contamination of meal. Moreover, there was no contact with farm animals.

The digestive tract could have been weakened by the cytomegalovirus primary infection, facilitating *G. anatis* translocation (CMV-seronegative patient before the graft). Virulence factors contributing to the pathogenicity of *G. anatis* have not yet been well defined, except for an atypical RTX (repeat in toxin) toxin, GtxA, responsible for the hemolytic activity and likely to be a major virulence factor (6, 9). Recently, a fimbrial GalF-A was also characterized as a key structure during colonization and invasion events of mucosal surfaces (10). This last structure could have been involved in digestive translocation leading to bacteremia. Indeed, GalF-A showed sequence similarity to the F17-like fimbrial protein precursor identified in the human extraintestinal pathogenic *Escherichia coli* (ExPEC) (11). Moreover, the infection has been likely favored by the immunosuppressive therapy overdose, the leucopenia, and the cytomegalovirus primary infection.

Like most environmental organisms, this bacterium was susceptible, except to fluoroquinolones and cotrimoxazole. The intrinsic or acquired resistance of *G. anatis* to antimicrobial drugs has not yet been elucidated. There has been much debate about the use of fluoroquinolones in veterinary medicine, but the proximity of *G. anatis* with poultry may be a hypothesis of this resistance. At last, the lack of commercially available biochemical gallery data-bases makes correct identification of this environmental organism difficult. It also underlines the usefulness of sequencing specific gene targets (16S rRNA genes, *sodA*, *hsp65*) for identification of unusual Gram-negative bacilli isolated from immunocompromised hosts (1). In the future, matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) mass spectrometry should allow more powerful and faster discrimination between species.

Nucleotide sequence accession numbers. The 16S and *sodA* nucleotide sequences of the isolate have been deposited in the GenBank database under accession numbers KF032910 and KF032911, respectively.

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