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CASE REPORT

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A case of human diarrhea caused by *Gallibacterium anatis*: a case report

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Abstract
Gallibacterium anatis (G. anatis) is an opportunistic pathogen previously associated with deaths in poultry and is also a pathogen that rarely causes human diseases. *G. anatis* has only been reported twice as the causative agent of a human disease (both in France). Here, we report a 62-year-old male patient with hypertension and type 2 diabetes who suffered from acute watery diarrhea caused by this bacterium which was identified by MALDI-TOF MS and 16 S rRNA sequencing. Despite human diarrhea caused by *G.anatis* is rare, with the continuous emergence of multidrug-resistant isolates of *G. anatis* in recent years, this case report will inform clinicians that *G. anatis* especially drug-resistant *G. anatis* may be a possible infectious source of human diarrhea in immune-suppressed populations.

Keywords *Gallibacterium anatis*, Infectious diseases, Diarrhea, Immune-suppressed populations, Diagnosis

Background

Gallibacterium anatis (*G. anatis*) is a Gram-negative bacterium of the *Pasteurellaceae* family [1]. *G. anatis* has been isolated from clinically healthy chickens as part of the normal microbiota in the upper respiratory and lower genital as well as digestive tracts [2]. Several studies in chickens revealed *G. anatis* to be an important bacterial pathogen associated with septicemia [3, 4]. These microbes result in reduced growth and loss in egg production in addition to causing chicken mortality [5]. Previous studies have shown that *G. anatis* not only infects a range of avian host species but also infects cattle, horses, pigs, sheep, and rabbits [5–7]. To date, *G. anatis* as a pathogen causing human diseases has only been reported twice (both in France) [8, 9]. Here, we report a case of diarrhea caused by this bacterium in a patient with hypertension and type 2 diabetes.

Case presentation

The case under consideration in this report is a 62-year-old man from Nantong City working in Sheyang Port, Yancheng City, Jiangsu Province. The patient had suffered from hypertension for 5 years and type 2 diabetes for 4 years. He followed a consistent and uniform weekday routine without habit of eating raw eggs and had no recent history of traveling abroad. Additionally, the patient had not come into contact with anyone who had diarrhea symptoms before experiencing symptoms. On the day before the onset of diarrhea, he purchased a chicken from a local farm and slaughtered it. After returning to the residence, he cooked and ate it with his four workmates. No one else had diarrhea.

On December 7th, 2022, the patient was admitted to the Emergency Intensive Care Unit (EICU) in our hospital, presenting with recurrent diarrhea for 5 days. Five days prior to admission, the patient suffered from acute

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watery diarrhea 7–8 times per day after catching a cold accompanied by nausea and vomiting, no feeling of urgency, no abdominal pain or bloating, and no fear of cold or fever. He took belladonna sulfamethoxazole and trimethoprim tablets (8 mg+400 mg+80 mg, 3 tablets, po tid) for 3 days by himself without the guidance of a doctor. However, there was no improvement. One day before admission, he visited the outpatient department of the Eighth People's Hospital of Tongzhou District, Nantong City. Laboratory tests showed elevated levels of white blood cells (WBCs) and eosinophils (Table 1). He received ciprofloxacin lactate (0.2 g iv. drip st) combined with clindamycin (1.5 g iv. drip st) as antimicrobial treatment, supplemented with famotidine (20 mg iv. drip st) for stomach protection, *Bifidobacterium* triple viable enteric-coated capsule (840 mg po bid) for regulating intestinal flora treatment and mixed glucose and electrolyte injection (500 ml iv. drip qd) for improving internal environment disorder. The patient did not have diarrhea in the afternoon but had diarrhea three times at night. Therefore, he sought treatment at our emergency department. He was admitted to the EICU (Emergency Intensive Care Unit). At admission, physical examination revealed a body temperature of 36.0 °C, pulse of 100 beats/min, respiratory rate of 18 breaths/min, and blood pressure of 110/77 mmHg, without joint pain. The results of stool laboratory examination were green watery stool, no mucus, no pus, no red blood cells (RBCs), no WBCs, no phagocytes, no parasite eggs, and no mold spores. Other laboratory tests showed elevated blood glucose (7.33 mmol/L), elevated WBCs and eosinophils (Table 1). Nucleic acid detection of 2019-Novel Coronavirus (2019-nCoV/SARS-CoV-2) by real-time reverse transcription-polymerase chain reaction (RT−PCR) was negative. The patient's stool was discharged into a disposable bedpan, then selected with a sterile cotton swab and inserted into Cary-Blair transport medium for culture. After taking the stool specimen for culture, the patient was given amoxicillin and potassium clavulanate for antimicrobial treatment (1.2 g iv. drip q8h). And given that the patient had high eosinophil count and might have allergies or eosinophilic enteritis, methylprednisolone succinate sodium ought to be supplemented as an anti-inflammatory agent (40 mg iv. drip qd).

Microbial culture and investigation of the patient's stool showed that there was no growth of Gram-negative bacilli on the Salmonella-Shigella agar plate. There were only smooth, moist, round, grayish, 1–2 mm in diameter, nontransparent colonies with a wide β-hemolytic zone on Columbia blood agar plates within 24–48 h of incubation at 35 °C and in a 5% CO_2_enriched atmosphere (Fig. 1A). Colonies were then identified as *G. anatis* by matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS, VITEK MS, VITEK MS IVD KB V3.0, bioMérieux, Lyon, France) with high confidence of 99.9%. To confirm the identification of this unusual bacterium in human clinical specimens, 16 S rRNA gene amplification and sequencing were performed

Laboratory tests	Normal range	One day before admission	First day of hospitalisation	Six days after admission
WBCs ($\times 10^9$/L)	4-10	21.48	18.68	9.04
Hemoglobin (g/L)	120-160	179	164	143
Neutrophil ($\times 10^9$/L)	2-7.70	10.78	7.84	5.57
Neutrophil percentage (%)	45-77	50.20	41.90	61.70
Eosinophil ($\times 10^9$/L)	0.05-0.50	5.75	6.40	0.60
Eosinophil percentage (%)	0.50-5	26.80	34.30	6.60
K (mmol/L)	3.50-5.50	/	4.06	3.72
Na (mmol/L)	135-145	/	137.10	138.30
Cl (mmol/L)	96-108	/	106.60	106.90
CRP (mg/L)	<10	8.70	2.08	3.28

Abbreviations: WBCs, white blood cells; CRP, C-reactive protein

Table 1 Results of laboratory tests of the patient at different times

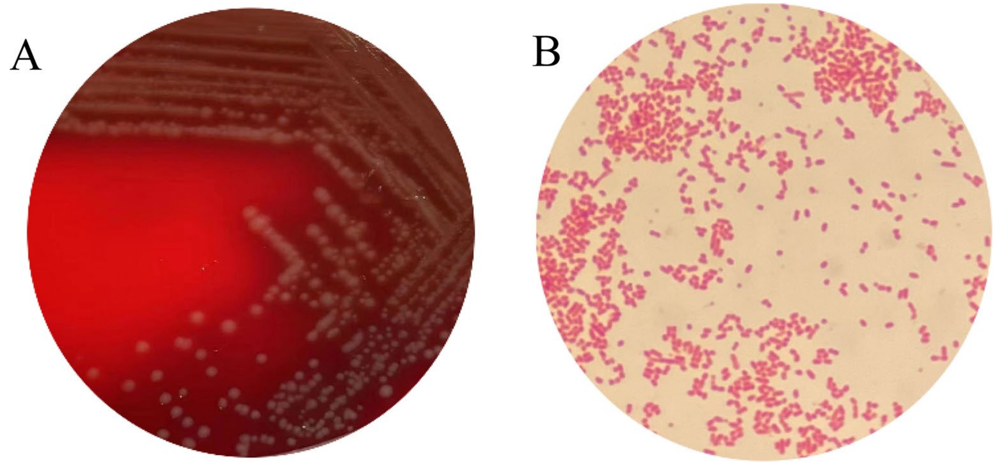

Fig. 1 Representative results of sample culture and Gram staining of bacteria. (**A**) Colonies on Columbia blood agar plate. (**B**) Gram-negative short-form bacilli of *G. anatis* NTSY 225,906 strain under microscope (Gram staining, ×1 000)

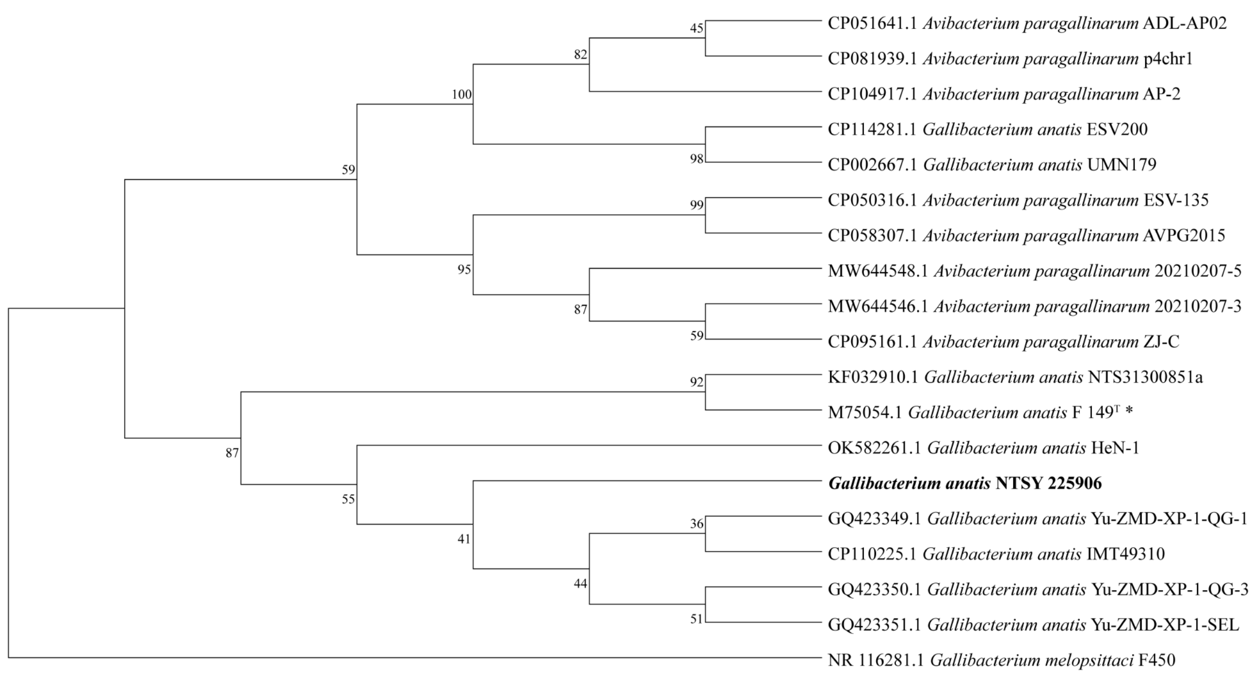

Fig. 2 Neighbour-Joining (NJ) phylogenetic tree based on 16 S rRNA gene sequence showing the relationships between *G. anatis* NTSY 225,906 and related taxa. *Gallibaterium melopsittaci* F450 (NR 116281.1) was used as an outgroup

with universal primers 27 F and 1492R as previously described [10]. The partial 16 S rRNA gene sequence (1412 bp, GenBank Accession No. OQ423124) shared highest identity to *G. anatis* (98.94–99.36%) and *Avibacterium paragallinarum* (94.14–94.39%) when matched to those in the GenBank database using the BLAST server. The sequence of French isolate (KF032910) [8] which shares 98.28% identity with our isolate(laboratory reference No. NTSY 225906). The neighbor-joining (NJ) [11] method was adopted to construct a molecular phylogenetic tree. The sequence of type strain F 149^T (=ATCC 43329^T=NCTC 11413^T) and the query cover 100% and percent identity 94.14 −99.36% sequences were selected as references from the list of BLAST. *Gallibaterium melopsittaci* F450 (NR 116281.1) was used as an outgroup. A phylogenetic tree (Fig. 2) was constructed using MEGA version 7.0 [12] based on 1000 replicates. The NJ phylogenetic tree showed that NTSY 225,906 formed a distinct cluster with *G. anatis*. This confirmed that the isolated strain in this study is *G. anatis.*

This bacterium grew on both Columbia blood agar and Mueller Hinton agar. It could not grow on MacConkey agar and Salmonella-Shigella agar. It is positive for catalase and oxidase. After Gram staining, the bacteria were

Drug	MIC (μg/mL)	Drug	MIC (μg/mL)
amikacin	≤8	ampicillin/sulbactam	≤4/2
gentamicin	≤8	piperacillin/tazobactam	≤4/4
imipenem	≤1	polymyxin	≤0.5
meropenem	≤1	trimethoprim/sulfamethoxazole	1/19
ceftazidime	≤1	chloramphenicol	≤4
cefepime	≤2	tetracycline	>8
cefotaxime	≤1	ampicillin	>16
aztreonam	≤2	ciprofloxacin	>2
piperacillin	8	levofloxacin	>8
moxicillin/clavulanic aci	≤4/2	moxifloxacin	>4

Table 2 Antimicrobial susceptibility testing results for *G. anatis* using the BD Phoenix TM-100 system

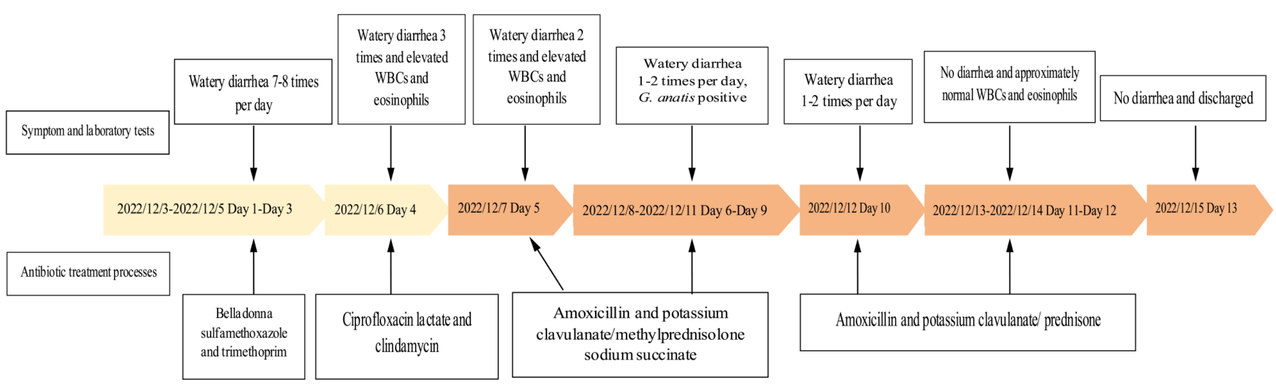

Fig. 3 Timelines of patient's diagnosis and treatment. Light and dark yellow represent the time before and after admission, respectively

single or paired, nonspore forming, Gram-negative short-form bacilli with blunt ends (Fig. 1B).

Concerning *G. anatis* species, no European Committee on Antimicrobial Susceptibility Testing (EUCAST) and Clinical and Laboratory Standards Institute (CLSI) specific recommendation for susceptibility testing exists. Therefore, in vitro susceptibility testing was performed using the BD Phoenix TM-100 system (Becton Dickinson Diagnostics, Sparks, MD, USA). Table 2 presents the minimum inhibitory concentration (MIC) values of this study. Therefore, there was no change in amoxicillin and potassium clavulanate antimicrobial drug treatment during hospitalization. Five days after admission, the dosage of hormone was changed to prednisone (30 mg po qd). During the first 6 days of hospitalization, the patient had watery stool diarrhea once or twice per day. Six days after admission, the patient had no diarrhea. Routine blood examination results were basically normal (Table 1). Eight days after admission, in consideration of the obvious improvement of the patient's symptoms, antimicrobial treatment with amoxicillin and clavulanate potassium was discontinued. The patient was cured and discharged from the hospital based on the discharge diagnosis of intestinal infection and hypereosinophilia. Details regarding the diagnosis and treatment are shown in Fig. 3.

Discussion

G. anatis is a mesophilic and facultatively anaerobic/microaerophilic, nonspore forming, nonmotile, and Gram-negative coccobacillus bacterium belonging to the family *Pasteurellaceae* [1, 13]. Currently, *Gallibacterium* consists of six different species, *G. anatis*, *G. melopsittaci*, *G. salpingitidis*, *G. trehalosifermentans*, *G.* genospecies

I-III, and *G.* group V [8, 14, 15]. The type strain of *G. anatis* is F 149^T (=ATCC 43329^T=NCTC 11413^T), isolated from the intestinal tract of a duck [1]. Recently, it was found that multidrug-resistant *G. anatis* isolates obtained from bronchoalveolar lavage (BAL) samples of 10 calves with bronchopneumonia unresponsive to antimicrobial therapy acquired resistance against 5–7 different antimicrobial classes [16]. *G. anatis* as a pathogen causing human diseases has only been described twice. It caused bacteremia and diarrhea in an immune-suppressed 26-year-old woman who received a bipulmonary transplant [8]. The second was isolated from the BAL fluid of a 71-year-old man with a lung abscess [9]. Here, we report the first case of human diarrhea caused by *G. anatis* in a patient with hypertension and type 2 diabetes. The patient we reported may have low immune response due to catching a cold and suffering from hypertension for 5 years and type 2 diabetes for 4 years. The highest blood pressure reached 200/110 mmHg.The patient was diagnosed with intestinal infection and hypereosinophilia. Differential diagnoses were required including chronic amoebic dysentery, rectal colon cancer, inflammatory bowel disease, and chronic dysentery. No amebic trophoblast or cyst was found in three independent stool samples under the light microscope. No *Shigella* bacteria were found during stool culture. Anti-nuclear antibody (ANA) and anti-neutrophil cytoplasmic antibody (ANCA) were all negative. Enteroscopy indicated a 3–4 mm small polyp in the ascending colon and transverse colon, respectively, with no ulcer or neoplasm. Based on these tests, the diagnoses of chronic amoebic dysentery, colorectal cancer, and inflammatory bowel disease were not supported, nor was there evidence of bacillary dysentery.

G. anatis has been divided into two phenotypically distinct biovars based on their hemolytic properties, hemolytic and nonhemolytic variants [1]. In contrast to the previously reported *G. anatis* clones without hemolysis on blood agar [8], the *G. anatis* colonies reported in this study had a wide β-hemolytic zone on Columbia blood agar. *Gallibacterium* toxin A (GtxA) is a secreted protein responsible for the hemolytic activity and leukotoxic properties of *G. anatis* and is considered the most important virulence factor of *G. anatis* [17, 18]. GtxA may play a role in pathogenesis [17] and induce the immune response of chickens [13]. We speculate that toxin GtxA may be the cause of elevated WBCs and eosinophils.

The *G. anatis* colonies reported in this study were susceptible to most antimicrobials tested, except to fluoroquinolones, ampicillin and tetracycline. Our results were in agreement with a reported study of *G. anatis* that was highly resistant to the antimicrobials enrofloxacin (90.5%) and tetracycline (76.2%) [19]. This results was also similar to the antibiotic resistance of *Gallibacterium anatis* biovar haemolytica isolates to ampicillin (73.1%), tetracycline (96.2%), enrofloxacin (88.0%) and ciprofloxacin (69.2%) in previous study [20]. The resistance of the isolates to tetracycline is typically caused by the tetracycline resistance genes *tetB*, *tetC* and *tetR*. The high drug resistance rate of fluoroquinolones may be due to the use of fluoroquinolones in veterinary medicine and mutations of quinolone targets within quinolone resistance determining regions (QRDRs), resulting in amino acid exchanges at positions 83 and 87 in GyrA (S83F, D87A) and ParC (S80I) [21, 22].

Conclusion

In conclusion, despite human diarrhea caused by *G. anatis* being rare, with the continuous emergence of multidrug-resistant isolates of *G. anatis* in recent years [16, 23], this case report will inform clinicians that *G. anatis* especially drug-resistant *G. anatis* may be a possible infectious source of human diarrhea in immune-suppressed population.

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Author contributions
HXW collected the data and drafted the manuscript. FW collected and analysed the data. HXH and JHZ collected the data. LPM undertook review and editing. All authors contributed to the article and approved the submitted version.

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Data availability
The datasets generated and analysed during the current study are available in the GenBank: OQ423124; https://www.ncbi.nlm.nih.gov/nuccore/OQ423124.

Declarations

Ethics approval and consent to participate
The study involving human participant was reviewed and approved by Ethics committee of Nantong Third People's Hospital, Affiliated Nantong Hospital 3 of Nantong University. The patient provided his written informed consent to participate in this study.

Consent for publication
Written informed consent was obtained from the patient for publication of this report and any accompanying images.

Competing interests
The authors declare no competing interests.

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