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First report of human infection by *Rhodoplanes* sp., Alphaproteobacteria in China

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ABSTRACT

We isolated a novel strain of Alphaproteobacteria from a patient, who had medical history of chronic rhinitis for more than twenty years and recently experienced local skin abscess and ulcer. He eventually died of multiple organ failure due to multi-antibiotics resistance. We identified the microorganism by 16SrRNA sequencing and found that it belonged to the genus *Rhodoplanes*. It was named as *Rhodoplanes* sp. strain ZLJ-0. It is resumed that *Rhodoplanes* sp. strain ZLJ-0 might be an emerging human pathogen involving in unknown febrile conditions and could cause local infection of any tissues or organs. Differential diagnosis of febrile patients should be conducted in clinical practice and research on emerging pathogens of Alphaproteobacteria should be performed to determine the epidemiology, clinical symptoms and pathogenic features of these pathogens.

1. Introduction

Alphaproteobacteria are widely found in the environment. *Legionella* is a confirmed pathogen of pneumonia[1,2] and *Parachlamydia* is an emerging human pathogen[3]. The etiology of others possible pathogens related to febrile infections is unknown. These bacteria are closely genetically- related to rickettisa and are difficulty to be cultured on common artificial medium. In 2005, with the help of the Collaborating Center for Rickettial Diseases (Marseille, France), WHO, as the national reference laboratory for rickettsiae and rickettsioses, our laboratory established new methods for culturing rickettsia, *e.g.* the shell vial method, which replaced the traditional chicken embryo culture previously used for surveillance of rickettsiosis. Since Oct. 2008, our laboratory has had collaboration with the Department of Infectious Disease, First Affiliated Hospital to Peking University in etiological investigations of unknown febrile patients. Recently, we isolated a species of Alphaproteobacteria from a patient, who had chronic rhinitis and recently experienced local skin abscess and ulcer.

2. Case report

A 49-year-old male had a progressive red swollen on the central flexure of the right thigh and recently developed ulcer (Figure 1). He had fever for 40 days prior to admission on Sep. 13, 2009 to the Department of Infectious Disease, First Affiliated Hospital of Peking University. The patient had chronic rhinitis for more than twenty years. He was febrile one or two times every year (>39 ℃) and treated by antibiotics (the names of medicine was unclear) treatment for one or two weeks. The patient denied the history of hypertension, diabetes, kidney disease, drug allergy or bleeding. He (as a leader of a labor union) and his colleagues went to the outskirts of Jiaozhou, Shandong Province to plant trees for about two weeks (the exact date was unclear) in April 2009. And he was not sure if he was bitten by any vector. After returning from the village he had a red swollen area in horsebean-size on the flexure central part of his right thigh and it was getting larger and larger although the surface of skin was intact. A painless bump with unclear boundary and elasticity in texture began to appear in size of a chicken egg. One month later, the

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patient became febrile (38 ℃) and took Roxithromycin and NSAIDs orally for about one week, but the symptom was not improved. He visited the outpatient at the local hospital on Aug. 12, and the laboratory examination showed WBC 2.12 ×10^9/L, NE 50.5%, PLT 187×10^9/L, ALT 275.7 U/L and AST 348 U/L, and total bilirubin 18.9 μmol/L. ACT result of the chest was normal, and ultrasonography of the abdomen showed the spleen was enlarged. Magnetic resonance imaging (MRI) displayed a subcutaneous soft-tissue nidus on the right thigh. A series of antibiotics including penicillin, levofloxacin, sodium cefoperazone, sulbactam, cefuroxime, ornidazole, cefepime, sodium imipenem/cliastatin and anti-infection herbal medicine were alternatively administrated but all were ineffective. The fever lasted and the patient became weaker. The derma ulcer nidus on the right thigh worsened forming a black eschar in the central part of the ulcer nidus. A general blood culture was performed but the result was negative.

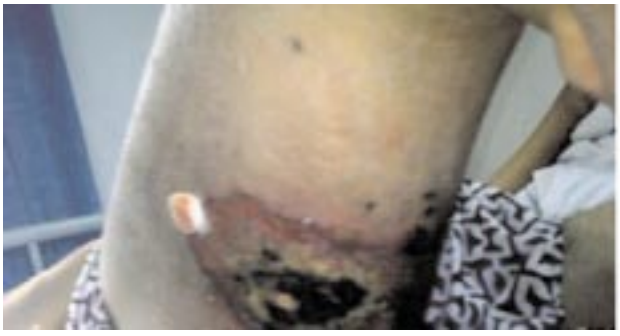

Figure 1. The derma ulcer nidus on the right thigh of the index patient.

On admission to the Department of Infectious Disease, First Affiliated Hospital of Peking University on Sep. 13, the physical examination showed 38.8 ℃ in body temperature and no skin rash. The abdominal examination showed palpable splenomegaly. An enlarge lymph node (4 cm × 5 cm) with tenderness was observed at the right groin area and an ulcer with escha was noted on the basolateral lymph node. On the central flexure and inside of the right thigh there was a large inflamed area with an ulcer and decaying tissue with some extravasate (Figure 1). Skin biopsy showed lymphocyte infiltration and small vassal dilation with erythrocyte extra-vasation. Pitting edema was observed on the right lower limb. Ultrasound of the abdomen showed multifocal inflammatory nidus of the liver. The laboratory data showed WBC 1.26 × 10^9/L, HB 102 g/L, PLT 31 × 10^9/L, PT 13.3 s, PA 66%, Rib-C 0.99g/L, D-D 2.35 mg/L, FDP 11.3 mg/L, ALT 236 IU/L, AST 366.2 IU/L, ALB 2.5 g/L, ALP 284 IU/L, total bilirubin 43.7 μmol/L, Crea 71 μmol/L, TG 2.59 μmol/L, TC 2.52 μmol/L, LDL-C 1 mg/L, K^+ 3.9 μmol/L, Na^+ 119.5 μmol/L, LDH 1 262 IU/L, CK 734 IU/L, CK-MB 1.4 ng/mL and PCT 0.65 ng/mL.

2 mL of blood with anti-coagulant sodium citrate was collected, out of which 0.5 mL blood was added into a Sell-Vial bottle (Cat No. 129AX/1, Bibby Sterilin Ltd., U.K.) and covered with a thin layer of L929 cells as reported previously[4]. After 5 days of culture, Gimenez stain and indirect immunofluorescence assay (IFA) were performed using the patient's serum. The result of light microscopy showed purple/red thin rod bacteria scattered outside and inside of the L929 cells (Figure 2A) Bacteria with green-yellow fluorescence was observed in and out of the cells by IFA (Figure 2B). The 16SrRNA gene of the isolates was amplified with primers Fd1 and rP2[5]. The sequencing demonstrated that this isolate had 99% nucleotide identity to the sequence of CRIB-02 16 SrRNA gene (accession No. DQ123619)[1] and only a thymine replaced uracil at position 77 in the CRIB-02. The isolate was named as ZLJ-0 and its 16 SrRNA gene sequence(GU247516) was deposited in the GeneBank. IgM and IgG antibody against the isolates was conducted by IFA and the titers of IgM and IgG were < 1:40 and 1:320, respectively. Because Alphaproteobacteria are often harbored within amoeba as amoeba-resisting bacteria (ARB), the IgG antibody against amoebae was also assayed using Entamoeba histolytica IgG ELISA diagnosis kit (IBL Co., Germany, Lot ENTG-032) according to the manufacturer's instruction and the result was negative. Detection of Alphaproteobacteria DNA by using primers Fd1 and rP2[5] and amoebae DNA using specific primers for Entamoeba histolytica P11 and P12[6] were performed and no positive result was observed.

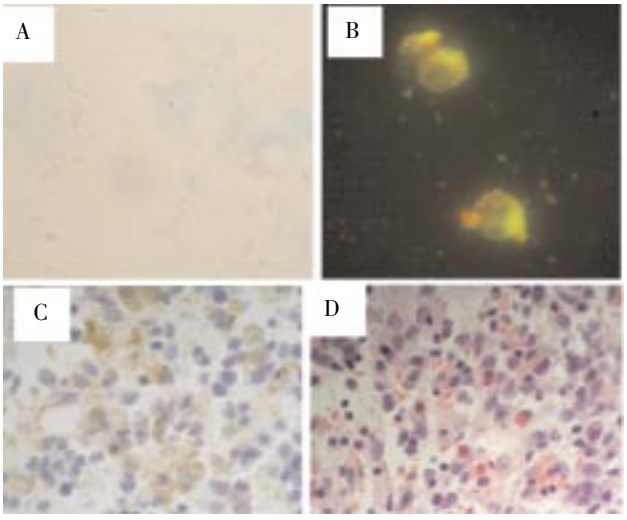

Figure 2. Bone marrow sections showing myelodysplas and hemophagocytic cells and the isolate of Alphaproteobacteria.
A: HE staining (1 000 ×) and B: CD68 staining (1 000 ×); C: Gimenez staining (1 000 ×) and D: immunofluorescence stain using the patient's serum, diluted 1:80 (600 ×).

Serial infection-related laboratory assays including the Weil-Felix reaction, Widals reaction and IgG antibody for Brucella showed negative.

On Sep. 14, ultrasonography showed severe soft tissue edema on the right thigh and enlarged lymphnodes in the groin areas. CT scanning indicated a moderately pleural effusion, intraperitoneal fluid and bowel lumen infiltration. The patient continued to be treated with antibiotics mentioned above alternatively but the fever was not abated.

On Sep. 17, the patient was treated with hydrocortisone that temporarily relieved the fever, but the fever relapsed with heart failure, arrhythmia, atrial fibrillation, paroxysmal tachycardia, an abnormal coagulation function, acid-base imbalance, and electrolyte disorder. Bone marrow examination was performed. The result of HE staining showed arrest of the bone marrow and CD68 staining showed increased hemophagocytic cells (Figure 2C) and principally the presence of hemophagocytic cells and myelodysplasia including hyperneutrophilia, hypererythrocytosis with myelocytes and metamyelocytes, and hypermegakaryoblasts with granular megakaryocytes (Figure 2D).

On Sep. 28, the patient began to cough, and auscultation

revealed severe rales and declining oxygen saturation. The relatives of the patient preferred to no further rescue and the patient died of pulmonary infections, respiratory failure, heart failure, arrhythmia, acute renal failure and acute entero-hemorrhaging. A temporally diagnosis of hemophagocytic syndrome was made.

3. Discussion

In this study, we reported the clinical characteristics of *Rhodoplanes* sp. infection based on etiological evidence. An Alphaproteobacteria strain was isolated by the Shell vial culture method from a patient, who had chronic rhinitis for more than twenty years and developed local skin abscess and ulcer leading to a multiple organ infection eventually. He died of multiple organ failure. The Gimenez stain of the isolate showed typical rickettsia-like features and its 16S rRNA gene sequence was 99% similar to strain CRIB-02 which belongs to the genus *Rhodoplanes* and was close to *Beijerinckia indica* subsp. lacticogenes (accession No. AJ563931). Recent reports show Alphaproteobacteria are amoeba-resisting bacteria (ARB) and are widely distributed in environmental samples such as domestic water systems or hospital water networks[6,7]. Further, these bacteria usually colonize free-living amoebae (FLA) and are resistant to most disinfectants due to amoebae protection.

The patient in this study experienced intermittent fever for about six months and had an original derma abscess, which later developed into ulcer nidus on the right thigh and then spread into multiple organs including the liver, kidney, lung, heart and brain. He eventually died of multiple organ failure. Diagnosis of hemophagocytic syndrome was clinically determined based on the observation of the marrow examination by HE and CD68 staining. The most significant clinical indicator is the multiple resistance of pathogen to antibiotics including penicillin, levofloxacin, sodium cefoperazone, sulbactam, cefuroxime, ornidazole, cefepime, sodium imipenem/cliastatin and anti-infection herbal medicine.

As we know, this is the first report of human case with *Rhodoplanes* sp infection in respiratory tract in China. Previously data show this bacterium was genetically related to the members of the order *Rickettsia* and widely present in the environment[8]. The Alphaproteobacteria group comprises two pathogenic agents, *Legionella*[9] and the emerging human pathogen-*Parachlamydia*[3]. Thomas suggested Alphaproteobacteria pathogens are potentially involving in nosocomial pneumonia with unknown etiology[10,11] and human infections may occur via inhalation of aerosols containing free bacteria[12]. Others suggest infected amoebae may be the infectious particles that bring bacteria to the lungs[13]. A new Alphaproteobacteria from hospital water supplies and nasal mucosa has been reported[14]. In this study, the patient had chronic rhinitis for more than twenty years and was in high fever (>39 ℃) one or two times every year in the onset of illness . Additionally, there was no evidence of a skin infection transmition due to injured skin although the patient had panted trees under poor hygiene for two weeks. The possibility of co-infection with amoebae was eliminated in the study because IgG antibody against amoebae was negative and specific 30 kDa protein gene of *Entamoeba histolytica* was not amplified by PCR. This *Rhodoplanes* sp causing a systemic infection disease exhibits resistance to multiple antibiotics. Similar pathogens may be involved with unknown etiology. The reason for few reports of these microorganisms is because the diagnosis equipment is scarce in hospitals and clinics. Tests have been developed as demonstrated, and researches on emerging pathogens of Alphaproteobacteria should be performed to determine the epidemiology, clinical signs and pathogenic features of these pathogens.

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Conflict of interest statement

We declare that we have no conflict of interest.

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