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Case Report

First report on the isolation of *Aureimonas altamirensis* from a patient with peritonitis

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SUMMARY

The isolation of *Aureimonas altamirensis* (a rare opportunistic pathogen with a yet unresolved pathogenicity) from the ascites fluid of a patient with bacterial peritonitis is reported. The strain was first identified by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) and the result was confirmed using 16S rDNA sequencing. An antimicrobial susceptibility profile was determined according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines published in 2013, revealing sensitivity to all antibiotics tested. The patient was treated effectively with levofloxacin.

1. Introduction

In 2006, Jurado et al. described a new bacterial species that was isolated from the subterranean environment of the Altamira cave in Cantabria, Spain.[1] The bacterium belonged to the genus *Aurantimonas* and was named *Aurantimonas altamirensis*.[1] These are Gram-negative, non-motile bacteria that grow in a strictly aerobic environment exhibiting a yellow colour. Biochemical testing has revealed positivity in catalase and urease, and oxidase reaction.[1] Furthermore, the ideal growing temperature has been determined to be between 10 and 40 °C with an optimum at 28 °C.[1]

Following taxonomic studies that included 16S rDNA sequencing and the comparison of fatty acid profiles, the genus *Aurantimonas* was reclassified in 2008 and renamed *Aureimonas*.[2] To date, only a few reports connect *Aureimonas altamirensis* with human infections. These bacteria have been isolated from the sputum of cystic fibrosis patients, but have also been involved in ophthalmologic diseases (keratitis) and have caused invasive infections (pleural effusion, bacteraemia).[3–5]

We report here, for the first time, the isolation of *A. altamirensis* in the ascites fluid of a 47-year-old woman suffering from a metastatic cancer of the cholangiocellular or pancreatic system, presenting with peritonitis.

2. Case report

In October 2013, a 47-year-old woman presented to the emergency department of the University Hospital Dresden with progressing ascites and vomiting. The patient's history showed a moderately differentiated adenocarcinoma (diagnosed in August 2012 in our institution). Histological analysis suggested that the primary tumour was either in the cholangiocellular system or in the pancreas. TNM staging revealed a grade IV tumour with metastasis in the liver and lymph nodes, as well as with peritoneal carcinomatosis. The initial treatment consisted of five courses of FOLFOXIRI (irinotecan 165 mg/m^2, oxaliplatin 85 mg/m^2, calcium folinate 200 mg/m^2, and 5-fluorouracil 3200 mg/m^2). To diminish the adverse affects we decided on an 80% dose reduction from the third course. Because of progressive disease we switched to cisplatin/gemcitabine (25 mg/m^2/1000 mg/m^2) in February 2013. However, re-staging in September 2013 showed advanced metastatic disease in the liver and peritoneum. Although the patient presented with grade I thrombocytopenia, grade III fatigue, and grade IV polyneuropathy, she requested the therapy to be continued. The therapy ended in November 2013.

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When the patient was first seen in the emergency department for the ascites (October 2013), chemotherapy was ongoing. She reported abdominal pain and nausea. Laboratory values showed a significantly elevated C-reactive protein (CRP) of 101.0 mg/l and a slight leukocytosis of 10.81×10^9/L. She underwent paracentesis, which removed 3 l of serous fluid. The ascites had an elevated white blood cell count of 0.74×10^9/l and an erythrocyte count of 0.003×10^{12}/L. Subsequently, the patient received oral levofloxacin. After the ascites puncture, the patient's physical condition improved and she returned home. Histological analysis of the ascites reported no evidence of malignancy, but microbiological analysis revealed bacterial peritonitis with the detection of *A. altamirensis*, sensitive to levofloxacin.

In November 2013 the patient was again admitted to the hospital due to recurrent ascites. Two weeks prior to this admission, chemotherapy had been discontinued because of the adverse effects described above and to continue with best supportive care. At this time, 5 l of fluid were removed. The cell count was normal and no malignant cells were found. Unfortunately, at this time point no material was sent to the Institute of Medical Microbiology. Recurrent increases in the ascites in this patient, who was in a palliative situation, led to the decision for permanent drainage of the ascites in order to avoid further hospitalization. The procedure was done in December 2013. Initially, 2 l ascites were removed every 2–3 days; the frequency and amount later decreased to 500–600 ml every 3 days. In January 2014, we were notified that the patient had died after having spent 2 months at home.

3. Microbiological analysis

Ten millilitres of ascites fluid was inoculated into the blood culture bottles and was sent to the diagnostic laboratory for microbiological analysis. They were incubated in an automated blood culture incubation system (BACTEC 9240; Becton Dickinson, Heidelberg, Germany) until positivity was indicated. The blood culture medium was then plated onto Columbia blood agar containing 5% sheep blood, chocolate agar, and bile–chrysoidin–glycerol agar for determination of bacterial growth. Additionally, Mueller–Hinton agar was used to obtain a preliminary resistance profile via agar dilution method. After incubation for 18 h at 37 °C and 5% CO_2, small yellow colonies grew on blood agar. For identification, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) (Bruker Daltonics, Bremen, Germany) was performed resulting in *A. altamirensis* (mass spectrometry score 1.927). However, since the interpretation of the result indicated no reliability at the species level, we conducted a 16S rDNA analysis. The BLAST algorithm (http://blast.ncbi.nlm.nih.gov) showed 100% homology with the respective 16S rDNA sequence deposited in the NCBI nucleotide collection (nr/nt). The antimicrobial susceptibility testing results were interpreted according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines published in 2013 (EUCAST clinical breakpoint table v. 3.1) using the non-species related breakpoints (Table 1).

Table 1
Antibiotic resistance profile using EUCAST breakpoints[a]

Antibiotic	MIC breakpoint, mg/l	Evaluation according to EUCAST
Ampicillin	0.032	S
Amoxicillin/clavulanic acid	0.016	S
Piperacillin/tazobactam	0.25	S
Cefotaxime	0.032	S
Imipenem	0.064	S
Meropenem	0.008	S
Ciprofloxacin	0.25	S
Gentamicin	0.25	S

EUCAST, European Committee on Antimicrobial Susceptibility Testing; MIC, minimum inhibitory concentration; S, susceptible.

[a] The antibiotic resistance profile was determined according to the clinical breakpoint table v. 3.1 published by EUCAST in 2013. Etest strips were used and the 'non-species related' breakpoints were used for interpretation. All tested antibiotics were effective against this *Aureimonas altamirensis* patient isolate.

4. Discussion

The use of mass spectrometry for routine diagnostics in the microbiology laboratory has become a major advantage for both routine applications and research purposes. The large number of reference spectra embedded in the MALDI-TOF MS database permits the highly confident identification of rarely occurring bacteria. In this present case, the recently described *A. altamirensis* was found by applying mass spectrometry. However, since the score value of 1.927 is too low to secure a reliable identification to the species level, we conducted 16S rDNA sequencing; this revealed a 100% homology to the *A. altamirensis* 16S rDNA sequences deposited in the NCBI nucleotide collection. We were therefore able to confirm the result from MALDI-TOF MS. Moreover, adding the main spectrum of our isolate to the MALDI-TOF MS database should lead to better identification for ongoing laboratory diagnostics.

The few case reports published on the involvement of *A. altamirensis* in human disease suggest its role as a causative agent of human infections.[3–5] A common characteristic of patients affected by this pathogen may be dysfunction of the immune system.[4,5] In our case, the patient was suffering from advanced cancer and was undergoing chemotherapy, thus assuming an impairment of the patient's immune system. However, the immune status of the three patients with *A. altamirensis* infection mentioned by Luong et al.[3] is not known.

For antimicrobial susceptibility testing, we chose antibiotics that are frequently used to treat Gram-negative bacteria and applied the guidelines for 'non-species related breakpoints' published by EUCAST in 2013 (http://www.eucast.org/clinical_breakpoints/). Our strain was susceptible to penicillins, cephalosporins, carbapenems, quinolones, and aminoglycosides. Interestingly, the minimum inhibitory concentration (MIC) values determined here were extremely low. We therefore propose that this bacterium has not been confronted with these antibiotics before and there was no need to develop or express antibiotic resistance mechanisms. However, it will be necessary to investigate a larger number of these strains to confirm this assumption.

In conclusion, we report, for the first time, the isolation of *A. altamirensis* from the ascites fluid of a cancer patient with bacterial peritonitis. The strain was initially identified using mass spectrometry, indicating the usefulness of this method for detecting rare pathogens. Our strain was susceptible to quinolones and the patient was treated effectively with levofloxacin. Future research needs to clarify the pathogenicity of *A. altamirensis* and its ability to cause infections in humans.

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Conflict of interest: No conflict of interest to declare.

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