JOURNAL OF CLINICAL MICROBIOLOGY, Aug. 2006, p. 3048–3050 Vol. 44, No. 8
0095-1137/06/$08.00+0 doi:10.1128/JCM.00521-06

First Report of Bacteremia by *Asaia bogorensis*, in a Patient with a History of Intravenous-Drug Abuse

Tamara Tuuminen,[1] Terhi Heinäsmäki,[2] and Tuija Kerttula[1]*

Division of Clinical Microbiology, Helsinki University Hospital, HUSLAB, Haartmaninkatu 3, P.O. Box 400, Fl-00029 HUS, Helsinki, Finland,[1] and Helsinki University Central Hospital, Department of Medicine, Division of Infectious Diseases, Helsinki, Finland[2]

Received 10 March 2006/Returned for modification 15 March 2006/Accepted 6 June 2006

We report the first documented case of bacteremia caused by *Asaia bogorensis* in a young patient with a history of intravenous-drug abuse. *A. bogorensis* was identified by sequencing the 16S rRNA gene. The isolate was exceptionally resistant to almost all antibiotics that are routinely tested for gram-negative rods but was susceptible to netilmicin, gentamicin, and doxycycline.

CASE REPORT

On 22 December 2005, a former immigrant from Estonia, a 23-year-old man, was transferred for consultation from the psychiatric outpatient department to the emergency care unit of the Helsinki University Hospital because the patient was suspected to have a systemic infection.

His past medical history included a Hill-Ewing sarcoma in 2002 that required resection and prosthetization and a chronic case of enterococcal arthritis in his operated knee between 2003 and 2005. A replacement of the prosthesis was considered impossible, and the patient was on continuous amoxicillin-clavulanate and clindamycin therapy. The adherence to this therapy was not optimal because of intravenous-drug abuse. He was put on a methadone substitution therapy in late 2005.

Upon arrival to the emergency care unit, the patient said that he had had a headache for 3 days and a fever had started the day before. His axillary temperature was 39°C, and he had an induration that was compatible with a recent injection in his right cubital fossa. His C-reactive protein (CRP) level was 117 mg/liter (normal, <10 mg/liter), that for leukocytes was 10E9/liter (normal range, 3.4E9 to 8.2E9/liter), and that for hemoglobin was 115 g/liter (normal range, 134 to 167 g/liter); trombocytes, electrolytes, creatinine, blood glucose, and liver function tests were within the normal range. Two consequent samples for blood culture were taken. The patient refused hospitalization and left the hospital at his own risk and on his regular antibiotic therapy.

Two days later, a gram-negative aerobic bacterium grew from both blood culture samples, showing an exceptionally resistant gram-negative bacterial strain which was difficult to identify by conventional bacteriological methods. The patient was contacted and advised to return to the hospital, which he managed to do on only the 6th day after the blood culture sampling.

Upon admission on December 28, his general condition was good and his axillary temperature was 37°C. His CRP level had spontaneously decreased to 45 (<10 mg/liter). The patient was hospitalized for 13 days and was treated, based on the preliminary antibiotic susceptibility reports, by intravenous tobramycin and ceftriaxone. His heart ultrasonography revealed neither endo- nor pericarditis, his chest X-ray was normal, and the result from ultrasonography of the upper abdomen was normal. He was discharged in a good condition with a normal CRP on his regular antimicrobial therapy.

Bacteriological examination was performed by routine collection of two sets of blood culture samples which were incubated in an automated blood culture system (BacT/Alert 3D; BioMérieux, Inc., Durham, NC). After 44.5 and 46 h of incubation, results from the two samples in aerobic bottles turned positive. A gram-negative rod, which was nonfermentative, strictly aerobic, oxidase negative, and catalase positive, was isolated. The isolate was subcultured on chocolate, horse blood, cystine-lactose electrolyte-deficient, and fastidious anaerobe agar media. Small colonies were already detectable after the overnight incubation on chocolate and blood agars that were incubated at both 30°C and 35°C in ambient air. On the second and third days, pale-rose, opaque, smooth colonies with entire margins were clearly visible by the naked eye on the chocolate agar, while it took more than 72 h to produce visible tiny colonies on the cystine-lactose electrolyte-deficient and MacConkey agars. The organism did not grow at all in anaerobic conditions. Gram staining revealed a gram-negative bacterium of variable length. Conventional biochemical test tubes checked on the next day showed the fermentation of glucose and arabinose, while results from other routine tests (indole, hydrogen sulfide, urease, citrate, *o*-nitrophenyl-β-D-galactopyranoside [ONPG], arginine dihydrolase, and motility) appeared negative. The biochemical characteristics of the isolate are presented in Table 1.

An identification of the isolate was attempted with several commercial identification systems. With the API20NE (BioMérieux, Inc., Marcy-l'Etoile, France), after 48 h of incubation, we obtained code 4045000. which gave significant taxa for *Pasteurella* spp., *Acinetobacter lwoffii*, *Aeromonas salmonicida* subsp. *masoucida*, and *Aeromonas salmonicida* subsp. *achromogenes*; however, the utilization of glucose and arabinose and the very slow utilization of mannose, the colony appearance, and the growth requirements spoke against the identification of these taxa. In an identification in a test panel for fermenta-

* Corresponding author. Mailing address: Division of Clinical Microbiology, Helsinki University Hospital, HUSLAB, Haartmaninkatu 3, P.O. Box 400, Fl-00029 HUS, Helsinki, Finland. Phone: 358 9 191 26893. Fax: 358 9 191 26244. E-mail: tuija.kerttula@hus.fi.

TABLE 1. Biochemical characteristics of *A. bogorensis* isolated from blood

Characteristic or test	Result (h)[b]
Growth on:	
Chocolate agar at 30°C and 35°C	Pos (o/n)
Horse blood agar at 30°C and 35°C	Pos (o/n)
CLED medium at 35°C	Pos (≥72)
MacConkey agar at 35°C	Pos (≥72)
Atmosphere	
Ambient atmosphere	Pos (o/n)
5% CO_2	Pos (o/n)
Anaerobic atmosphere	No
Fermentation of:	
Glucose	Pos
Arabinose	Pos
Mannitol	Neg
Inositol	Neg
Sorbitol	Neg
Rhamnose	Neg
Sucrose	Neg
Melibiose	Pos
Utilization of:	
Mannitol	Pos
Citrate	Neg
Glycerol[a]	Pos
Production of:	
Indole	Neg
Hydrogen sulfide	Neg
Enzyme activity	
Arginine dehydrolase	Neg
Ornitine decarboxylase	Neg
Lysine decarboxylase	Neg
Urease	Neg
Other reactions	
β-Galactosidase (ONPG)	Neg
Pigmentation	Pale rose
Motility	Neg
Oxidase test	Neg
Catalase test	Pos

[a] Tested with ID32C (BioMérieux, Inc.).
[b] Pos, postive; Neg, negative; o/n, overnight incubation.

TABLE 2. Antibiotic susceptibility of the clinical isolate *A. bogorensis*

Antibiotic	MIC (mg/liter)
Amikacin	24
Amoxicillin-clavulanate	24
Ampicillin-sulbactam	32
Aztreonam	>256
Cefepime	>32
Cefotaxime	>32
Ceftazidime	64
Ceftriaxone	12
Cefuroxime	24
Ciprofloxacin	>32
Colistin	>1,024
Doxycycline	1.5
Gentamicin	3
Levofloxacin	>32
Meropenem	>32
Netilmicin	1.5
Penicillin	>32
Piperacillin-tazobactam	>256
Tigecyclin	3
Tobramycin	8
Trimethoprim-sulfamethoxazole	>32

tive bacteria, the API20E (BioMérieux, Inc.) suggested a typical code for *Acinetobacter baumannii*, i.e., 0004042. This taxon could not be accepted due to the exceptional resistance to colistin, a morphology different from that of *A. baumannii* by Gram staining, different growth requirements, and the pale-rose pigmentation of the colonies. When analyzing with the Rapid NF Plus (Remel, Inc., Lenexa, Kansas), we obtained code 650362, which suggested *Stenotrophomonas maltophilia*, but the probability of identification level was questionable. This taxon could not be accepted either.

The final identification of the bacterium was achieved by sequencing the 16S rRNA gene. An extraction of bacterial DNA from bacterial cells was performed by heating (100°C for 10 min). DNA was amplified essentially as described previously (1). The amplification was performed with the forward primer fD1mod, 5′-AGAGTTTGATC(T/C)TGG(T/C)T(T/C)AG-3′, and the reverse primer 533R, 5′-TTACCGCGGCTGCTGGC AC-3′, and yielded a 525-bp product, the quality of which was checked in an agarose gel. The product was then sequenced using MegaPace 100 (Amersham Bioscience) in a reverse direction using the 533R primer. The obtained sequence was compared to those in the database at http://www.ebi.ac.uk /fasta33/. It shared 99.721% identity with the corresponding *Asaia bogorensis* isolate deposited under GenBank accession no. AY368591 and 99.164% identity with *Asaia bogorensis* deposited under GenBank accession no. AB025928. In our laboratory, it was given the designation T-50271.

Since this organism has not been recognized as a human pathogen, there are no established susceptibility guidelines. However, to support clinicians in their choice of antibiotic treatment, we applied our routine susceptibility testing methods, which are in accordance with CLSI guidelines. The isolate did not grow on Mueller-Hinton agar, which is routinely used in our laboratory for susceptibility analysis of coliform and nonfermentative pseudomonas-like bacteria. On the contrary, it grew well on Haemophilus test medium, which was used instead. The antibiotic susceptibility was determined with the agar gradient diffusion method, i.e., the Etest (AB Biodisk, Solna, Sweden). The bacteria showed exceptionally high MICs to almost all antibiotics that are routinely tested for gram-negative rods in our clinical laboratory, while MICs for netilmicin, gentamicin, and doxycycline were markedly lower, suggesting susceptibility to these antibiotics. The MICs for the isolate are listed in Table 2.

Asaia bogorensis is a recently identified bacterium (4), and the *Asaia* genus at present comprises three species. The natural habitat of *Asaia* is tropical flowers and fruits of the Southeast Asia region. Contamination of beverages with *Asaia* may result in spoilage, as reported by Moore et al (2). In several interviews with the patient, the source of the bacterium remained unknown; however, it is tempting to speculate that some narcotic substances might have been contaminated by

Asaia. This organism, being nonpathogenic, might have silently resided attached to the biofilm on the prosthesis for several years until the recent episode of bacteremia.

Etests on Haemophilus test medium were applied to determine the antibiotic susceptibility profile. The MICs for all analyzed β-lactams, carbapenems, monobactam, and fluoroquinolones were high, ranging from 12 to over 256 μg/ml, suggesting extended resistance of the bacterium to most antibiotics, a phenomenon which was described earlier (3). However, the bacterium appeared to have an apparently lower MIC for doxycycline and MICs to aminoglycosides were variable, being lowest to netilmicin and gentamicin and substantially higher to tobramycin and amikacin. In earlier studies (3), the bacterium was found to be susceptible to amikacin and tobramycin. We, too, at first obtained lower MICs to tobramycin and ceftriaxone. However, on second testing and probably using a more accurate inoculum, MICs were slightly higher and the isolate was interpreted as being less sensitive to both tobramycin and ceftriaxone.

A PubMed search revealed only one earlier-reported clinical case of *A. bogorensis* infection; this was in a 41-year-old woman with end-stage renal disease who was on peritoneal dialysis for 5 years (3). Our patient did not experience severe inflammation-related syndrome. On the contrary, he tolerated his bacteremia well. The reported case of *A. bogorensis* peritonitis was, however, complicated by septic features such as a drop in blood pressure and tachycardia. The sources of bacteria in our case and the earlier-reported case remained enigmas. However, there are several common features in the two cases. Both involved chronic underlying diseases, an indwelling foreign body, and several preceding deep infections caused by nosocomial bacteria. Another common feature between our patient and the earlier-reported patient was that *A. bogorensis* was cultivated despite prophylactic antibiotic coverage: cefepime and ampicillin-sulbactam in the peritonitis patient and amoxicillin-clavulanate and clindamycin in our patient. Yet another common feature between our case and the earlier-reported case was that the final identification of the bacterium was done by means of a 16S rRNA sequence analysis.

With an increasing use of indwelling foreign devices and implants or abuse of intravenous narcotic substances, clinicians and pathologists may come across exotic bacteria not previously described as human pathogens. As this kind of environmentally acquired bacterium is typically very difficult to identify with the routine identification methods used in clinical laboratories, we, like Snyder et al. (3), appreciate new molecular techniques, such as 16S rRNA sequencing, which allowed the identification of this unusual bacterium from the patient's blood culture.

We thank Martti Vaara, the Head of the Clinical Microbiology Department at the HUSLAB, for his valuable advice during our investigation and manuscript preparation.

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