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First Report of Bloodstream Infection Caused by *Pseudomonas fulva*▽†

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***Pseudomonas fulva* has not yet been isolated from humans as a pathogen. Herein, we report the first case of *P. fulva* bacteremia in a patient hospitalized due to trauma. The species was identified using biochemical and molecular genetic analyses of the 16S rRNA, *gyrB*, *rpoB*, and *rpoD* genes.**

CASE REPORT

A 56-year-old man who was seriously injured in an accident at a construction site was admitted to the emergency department. He had emergency surgery for femur fracture and femoral artery rupture and received amikacin and cefotiam on the same day. On hospital day 2, he developed a fever (38.2°C) and leukocytosis (white blood cell count, 11.5×10^9/liter), with 89% of the cells being polymorphonuclear leukocytes, and the C-reactive protein (CRP) level was 128 mg/liter. Three sets of blood samples and a wound swab were obtained for cultures, which were negative for growth. After the first operation, the patient suffered from rhabdomyolysis and compartment syndrome. Consequently, an above-the-knee amputation was performed on day 3. One day after the second operation, his body temperature suddenly further increased to 39.5°C, and three sets of blood samples were drawn for additional cultures. After one day of incubation in the BacT/Alert 3D blood culture system (bioMérieux, Marcy l'Etoile, France), three aerobic and two anaerobic culture bottles registered positive for bacterial growth. Gram staining morphologies of the broths from the aerobic and anaerobic culture bottles were identical, showing Gram-negative bacilli. A single morphotype of medium-sized, round, convex, and yellowish-brown-colored colonies on sheep blood agar and small-sized, round, and colorless colonies on MacConkey agar was obtained after subcultures from the three aerobic bottles. All colonies showed identical triple sugar iron reaction results of the alkaline slant-butt reactions and were negative for gas and hydrogen sulfide production.

The Vitek 2 GN and ID 32 GN systems (bioMérieux) were used for species identification. Both profiles were suggestive of *Pseudomonas putida* (99.0% and 99.9% probabilities, respectively). However, interestingly, the isolate grown on MacConkey agar showed a negative oxidase reaction, and colonies on blood agar showed a weakly positive oxidase reaction. We designated the isolate YMC09/4/B4619 by its specimen number, and we subjected it to 16S rRNA gene sequence analysis. DNA was extracted using a DNeasy blood and tissue kit (Qiagen GmbH, Hilden, Germany). The universal primers 8F (5′-AGA GTT TGA TCC TGG CTC AG-3′) and 1541R (5′-AAG GAG GTG ATC CAG CCG CA-3′) were used to amplify a 1,395-bp segment corresponding to part of the 16S rRNA gene (6). The obtained sequence was compared with all 16S rRNA sequences available in the EMBL Nucleotide Sequence Database by using the FASTA program (http://www.ebi.ac.uk/fasta33/). The highest sequence identity value of 99.7% (1,392/1,395 bp) was obtained with the strains of *Pseudomonas fulva* (NRIC 0180^T; GenBank accession number AB060136). The next highest identity of 99.6% was obtained with *P. putida* strains (NCB0308-456; GenBank accession number AB294558). Additionally, we subjected it to *gyrB*, *rpoB*, and *rpoD* gene sequence analyses. In brief, the designed primers gyrb 2f (5′-TCC GAG CAA CTG ATA CTG ACG-3′), gyrb 2r (5′-GCC TTT ACG GCG AGT CAT CT-3′), rpob 1f (5′-TCA AGG AAC GTC TGT CGA TG-3′), rpob 1r (5′-GTT CGG GAT GTC TGC AGT G-3′), rpod 1f (5′-AGC TGC TGA CCC GTG AAG-3′), and rpod 1r (5′-TTC CTT GAT TTC GGA AAC G-3′) were used to generate amplicons of each gene sequence. Amplicons of 666 bp (*gyrB*), 1,034 bp (*rpoB*), and 723 bp (*rpoD*) were produced. The sequences of the strain YMC09/4/B4619 were found to be most closely related to those of the type strains of *P. fulva*; identity was 92.5% with strain IAM 1529^T (GenBank accession number AB039395) for *gyrB* gene, 97.2% with strain CIP 106765^T (GenBank accession number AJ717419) for *rpoB* gene, and 96.5% with strain IAM 1529 (GenBank accession number AB039586) for *rpoD* gene. The identities with *P. putida* strains were 89.1% with strain ATCC17485, 93.4% with strain KT2440, and 89.1% with strain ATCC17485 for *gyrB*, *rpoB*, and *rpoD* genes, respectively.

Physiological and biochemical characteristics of the strain YMC09/4/B4619 were determined using conventional methods and miniaturized identification systems. Production of pyocyanin and formation of fluorescent pigments were tested on *Pseudomonas* agar P and *Pseudomonas* agar F (Difco Laboratories, Detroit, MI), respectively. The motility test results, oxidase and catalase reactions, test results for indole, and hydrolysis of starch were determined. The growth at 4 and 41°C was determined on Mueller-Hinton agar (BBL, BD Diagnostics,

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Sparks, MD) (1). Vitek 2 GN, ID 32 GN, and API 20NE systems (bioMérieux) were used to test biochemical properties according to the manufacturer's instructions. Phenotypic characteristics are displayed in Table S1 in the supplemental material. It was motile and positive for production of yellow-orange pigments, catalase production, arginine dihydrolase, and growth at 4°C. The strain utilized D-glucose, D-mannose, gluconate, malate, and citrate. It was negative for the production of fluorescent pigment and pyocyanin, hydrolysis of gelatin and starch, nitrate reduction, growth at 41°C, and utilization of maltose, sucrose, D-mannitol, malonate, and *m*-hydroxybenzoic acid.

Phylogenetic trees were constructed to confirm the species not only from the data of the 16S rRNA (8) but also from the combined nucleotide sequences of the *gyrB* and *rpoD* genes, with the assumption that longer sequences would result in better reliability (9). The phylogenetic trees revealed that the strain YMC09/4/B4619 had the highest sequence similarity to *P. fulva* sequences (see Fig. S1 in the supplemental material).

Antimicrobial susceptibility of the organism was determined using Etest (AB Biodisk, Solna, Sweden) according to the manufacturer's instruction. The antibiotic susceptibility patterns were interpreted according to Clinical and Laboratory Standards Institute standards (2). The organism was resistant to chloramphenicol and trimethoprim-sulfamethoxazole and was susceptible to piperacillin-tazobactam, ceftazidime, cefotaxime, aztreonam, cefepime, imipenem, meropenem, gentamicin, tobramycin, amikacin, and levofloxacin.

The patient was treated with piperacillin-tazobactam and clindamycin after his fever spiked on day 4. His body temperature decreased to 37°C on day 6. Follow-up blood cultures were requested on day 7, and results thereafter were negative for growth. Four environmental cultures of soil and instruments from the accident site were performed, but they showed no organism growth. The patient was discharged on day 76 after he underwent rehabilitation training.

Pseudomonas fulva was isolated for the first time from Japanese rice paddies in 1963 (8). It has not yet been isolated from humans as an infectious agent, and its association with human infections was otherwise unknown. This is the first report in which *P. fulva* has been isolated from human blood samples.

Pseudomonas fulva shares phenotypic characteristics with members of the fluorescent *Pseudomonas* group, particularly *P. putida*, but possesses some unique features. Most remarkably, *P. fulva* produces yellow pigments, whereas it does not produce fluorescent pigment, unlike *P. putida*. And *P. fulva* does not assimilate malonate and *m*-hydroxybenzoate, whereas *P. putida* does. It is also different from *Pseudomonas aeruginosa* in its inability for growing at 41°C, reducing nitrate, hydrolysis of gelatin, and utilization of D-mannitol (7, 8) (see Table S1 in the supplemental material). Uniquely, its oxidase reaction is weakly positive and may be negative when taken from the surface of a differential medium, such as MacConkey agar, due to acidification resulting from the fermentation of carbohydrates (4). Some authors report that 16S rRNA gene sequences are too conservative to determine inter- and intrageneric relationships in closely related bacterial species (3). Notably, the *gyrB* gene has been reported to be a more reliable PCR target than the 16S rRNA sequences for detection of *P. aeruginosa* (5). However, in this case, identification of the isolate was confirmed by the congruent results of genetic analyses of the 16S rRNA, *gyrB*, *rpoB*, and *rpoD* gene sequences. The anaerobic broth was not cultured because the Gram stain morphologies of both the aerobic and anaerobic culture bottles were identical. Nonetheless, anaerobes might be involved in this infection.

Pseudomonas fulva is isolated mainly from environmental sources. Although environmental cultures from the accident field were negative, we hypothesize that the bacteria likely entered the bloodstream through the wound inflicted at the construction site. We believe that from now on *P. fulva* should be considered another potential microorganism of trauma-related bloodstream infections in humans.

Nucleotide sequence accession numbers. The nucleotide sequence data of the strain YMC09/4/B4619 have been assigned EMBL/GenBank nucleotide accession numbers FN599522, FN599523, FN599524, and FN599525 for the 16S rRNA, *gyrB*, *rpoB*, and *rpoD* genes, respectively.

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