

Clinical Characteristics of *Ochrobactrum anthropi* Bacteremia

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The clinical picture of *Ochrobactrum anthropi* infection is not well described because the infection is rare in humans and identification of the pathogen is difficult. We present a case of *O. anthropi* bacteremia that was initially misidentified as *Ralstonia paucula* and later identified by 16S rRNA sequencing and *recA* analysis.

CASE REPORT

An 85-year-old man was admitted to our hospital to receive transcatheter arterial chemoembolization (TACE). He had liver cirrhosis (Child-Pugh score A) and hepatocellular carcinoma (HCC) caused by hepatitis C virus infection. His medical history included repeated radio frequency ablation and a total of nine TACE procedures for the treatment of HCC. He had also experienced urinary bladder and ureteral carcinoma.

The patient was free from any complications; however, he suddenly developed high fever (39°C), chills, and rigors 12 days after TACE. Physical examination did not reveal any significant findings, but laboratory testing showed a highly inflammatory state: his white blood cell count was 17,700/mm³, and his C-reactive protein level was 4.5 mg/dl. Urine examination was normal, and whole-body computed tomography revealed no particular pathogenic lesion. An echocardiogram was not performed. Oral cefcapene pivoxil was prescribed for 1 week based on the suspicion of some forms of infection relating to TACE.

Two sets of blood cultures were obtained at the time of high fever. Of these four bottles, two different aerobic bottles became positive for Gram-negative rods after an incubation period of 36 h. However, the patient's condition and laboratory measurements improved promptly, and he was discharged on day 16 without distinct diagnosis. Later, the organism was identified as *Ralstonia paucula* by the Microscan walkaway system (Siemens) with a concordance rate of 99.9%. Susceptibility testing showed the organism to be sensitive to imipenem, meropenem, amikacin, minocycline, and colistin but resistant to β-lactams such as piperacillin, piperacillin-tazobactam, ceftazidime, cefepime, aztreonam, levofloxacin, ciprofloxacin, gentamicin, and trimethoprim-sulfamethoxazole. Although the pathogen was resistant to the antibiotic prescribed, he responded well to the treatment and the antibiotic did not need to be changed.

The organism had been rarely encountered at our hospital, and therefore the sample was sent to Kochi University for further identification. The two samples derived from different blood cultures were examined separately. Blood samples were inoculated onto agar media and incubated at 28°C. Pale yellow colonies grew in culture and released a popcorn-like smell. Almost the entire region of the 16S rRNA gene was amplified by colony PCR using a pair of primers (pr0R2, 5'-AGAGTTTGATCMTGGCTCAG-3'; 9Rev, 5'-AAGGAGGTGATCCAGCC-3') and sequenced with the BigDye Terminator v3.1 cycle sequencing kit. The resulting sequences from the two samples were identical. BLAST analysis against the NCBI database revealed the organism not to be *R.*

paucula but rather to be of the *Ochrobactrum anthropi* cluster, which is highly homologous to *Brucella* spp. (Fig. 1). The 16S rRNA partial sequences of isolated organisms are 100% identical to those of the *O. anthropi* cluster (Fig. 1). Subsequently, almost the entire region of the *recA* gene was amplified by colony PCR with a pair of primers (1) (*recA*-BrucOchro-f, 5'-ATGTCTCAAAAATTCATTGCGAC-3'; *recA*-BrucOchro-r, AGCATCTTCTTCCGGTCCGC-3') and sequenced. Again, two colonies had the same sequences and were 100% identical to *recA* of several *O. anthropi* strains (Fig. 2), confirming that the organism is *O. anthropi*.

Ochrobactrum spp. belongs to the *Brucellaceae* and is known to be isolated from *Leguminosae* nodules (2, 3). Its name is derived from the Greek *ochros*, meaning pale yellow; this is the characteristic color of *Ochrobactrum* colonies. The organism has the potential to colonize an exceptionally wide variety of habitats (4–8). The genus *Ochrobactrum* currently comprises 9 species; to date, only 3 species, *O. anthropi*, *O. intermedium*, and *O. pseudintermedium*, have been reported to occur in clinical samples (9).

Of the clinically relevant species, *O. anthropi* is becoming increasingly recognized as a potentially problematic, opportunistic, and nosocomial pathogen (10, 11). *O. anthropi* is an aerobic, oxidase-positive, urease-positive, Gram-negative, motile, non-lactose-fermenting bacillus previously known as “*Achromobacter* group Vd” (12). Most cases of human disease due to this pathogen that have been reported have been associated with central venous catheter line infection (13–18). However, this organism has also been reported as a cause of infective endocarditis (10, 19, 20), pancreatic abscess (21), puncture wound osteochondritis (22), endophthalmitis (23), urinary tract infection (24), meningitis (25), pelvic abscess (26), and osteomyelitis (27). Infection is most commonly, although not exclusively, seen in immunocompromised patients, such as those with debilitating illnesses or malignancy (13, 14, 19, 26, 28–30). Opportunistic infections and nosocomial outbreaks of *O. anthropi* are being increasingly reported.

Yu et al. reported the clinical characteristics of 15 cases of *O.*

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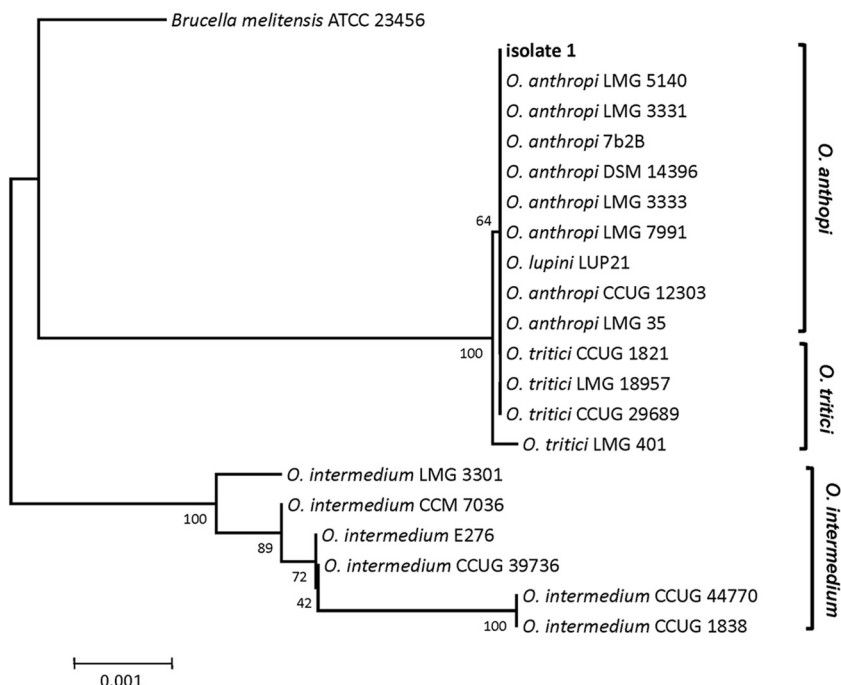


FIG 1 Phylogenetic tree of partial 16S rRNA nucleotide sequences (1,430 nucleotides) using neighbor-joining analysis.

anthropi bacteremia (31). They stated that all patients had severe underlying disease and manifested primary *O. anthropi* bacteremia without obvious focus; however, none of the patients died directly from the infection. Thus, although *O. anthropi* can be pathogenic in critically ill or immunocompromised patients, the organism is considered to be of relatively low virulence. However, the clinical picture of *O. anthropi* infection, especially bacteremia,

has still not been well described. The reason is the difficulty of differentiating *Ochrobactrum* spp. from other organisms by physiological tests because of their high phenotypic similarity (32). To gain a better understanding of the clinical manifestations of *O. anthropi* infection, we need to be able to clearly identify the pathogen.

Commercially available test systems used in routine diagnosis are not suitable for species discrimination within the genus *Ochro-*

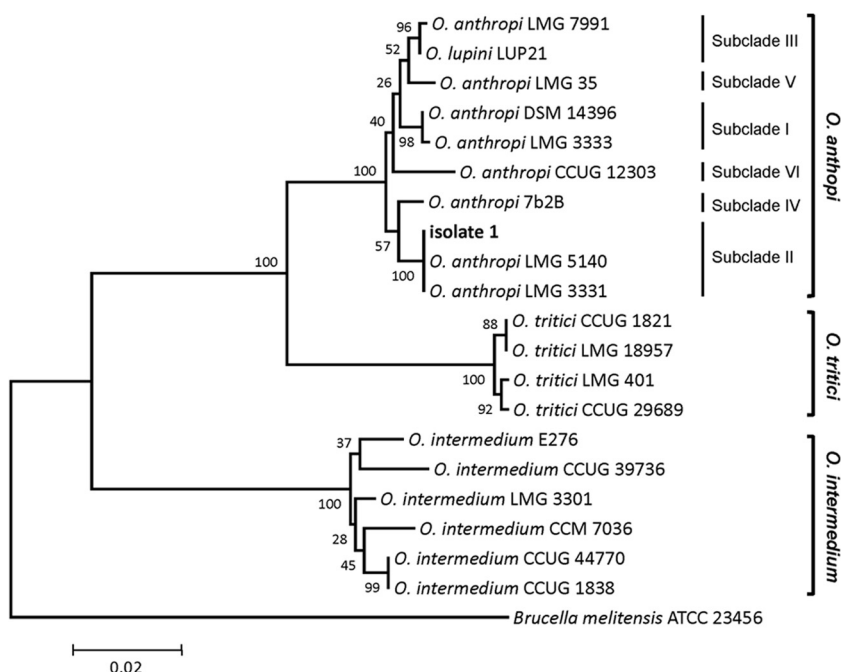


FIG 2 Phylogenetic tree of partial *recA* nucleotide sequences (897 nucleotides) using neighbor-joining analysis.

TABLE 1 Comparison of the biochemical properties of *Ochrobactrum anthropi*, *Ralstonia paucula*, and the isolate in the present case

Property ^a	Result ^b for		
	<i>Ochrobactrum anthropi</i>	<i>Ralstonia paucula</i>	Isolate in the present case
URE	10	5	(+)
H ₂ S	1	1	(-)
IND	1	1	(-)
LYS	1	1	(-)
ARG	5	1	(-)
ORN	1	1	(-)
TDA	25	1	(-)
ESC	1	1	(-)
VP	1	1	(-)
CIT	75	75	(-)
MAL	10	75	(+)
ONPG	1	1	(-)
TAR	5	50	(-)
ACE	1	25	(+)
CET	1	10	(-)
OF/G	99	10	(+)
P ₄	99	90	(+)
K ₄	99	90	(+)
CL ₄	50	25	(-)
FD ₆₄	95	90	(+)
TO ₄	25	75	(-)
NIT	95	10	(-)
OXI	99	99	(+)

^a All biochemical properties were derived from the MicroScan system (Siemens). URE, urea; H₂S, hydrogen sulfide; IND, indole; LYS, lysine; ARG, arginine; ORN, ornithine; TDA, tryptophan deaminase; ESC, esculin; VP, Voges-Proskauer reaction; CIT, citric acid; MAL, malonic acid; ONPG, *o*-nitrophenyl- β -D-galactopyranoside; TAR, tartaric acid; ACE, acetamide; CET, cetrinide; OF/G, oxidation-fermentation test/glucose; P₄, resistance rate to penicillin G 4 μ g/ml; K₄, resistance rate to kanamycin 4 μ g/ml; CL₄, resistance rate to colistin 4 μ g/ml; FD₆₄, resistance rate to nitrofurantoin 64 μ g/ml; TO₄, resistance rate to tobramycin 4 μ g/ml; NIT, nitrate reduction; OXI, oxidase.

^b The numbers represents the estimated percentages of the positive rates. 1, very rare for positive reaction; 5, rare for positive reaction; 10, usually not a positive reaction; 25, mostly negative reaction; 50, positive or negative reaction; 75, usually positive reaction; 90, mostly positive reaction; 95, almost all are positive reaction; 99, positive reaction.

bactrum. In fact, the pathogen in the present case was first misidentified as *R. paucula*. Although the biochemical properties of *O. anthropi* and *R. paucula* are similar, they have distinct differences, especially regarding malonic acid (MAL), the oxidation-fermentation test with glucose (OF/G), tobramycin (TO₄), and nitrate reduction (NIT) reactions (Table 1). Of these, a positive reaction with MAL and a negative result with NIT were assumed to be the major reasons for misidentification in this case, while the results of OF/G and TO₄ were rather consistent with those expected for *O. anthropi*.

Misidentification of *O. anthropi* as other members of the *Brucellaceae* has already been reported (33). At present, 16S rRNA gene sequencing is used for the identification and differentiation of *O. anthropi* and *Brucella* spp. However, this approach, in particular partial sequencing, is prone to misidentifying these pathogens because of their high sequence similarities (9). Meanwhile, *recA* analysis provides more accurate identification and differentiation (9, 34).

In the present case, the pathogen was first identified as a member of the *O. anthropi* cluster by 16S rRNA analysis (Fig. 1). At that point, the pathogen could not be completely differentiated from

other *Ochrobactrum* spp. such as *O. lupini* (1, 35). Since there are few clinical reports concerning infections with other *Ochrobactrum* spp., further investigation was required for identification. Eventually, the pathogen was successfully matched and confirmed as *O. anthropi* subclade II by *recA* analysis (34) (Fig. 2).

As discussed, catheter line infection is the most common way by which *O. anthropi* causes infection in humans (13–18). However, our patient had not received a central venous line or an arterial line during his hospitalization. Physical examination and systemic investigation did not reveal any symptoms or findings, and considering his clinical course, it was quite unlikely that infective endocarditis existed although an echocardiogram was not performed. The possibility of blood culture contamination was extremely low since genetically identical organisms were detected from separately obtained blood cultures. The only possible focus of infection was assumed to be a peripheral intravenous line, i.e., peripheral line-associated bloodstream infection; however, it could be a primary bacteremia as reported elsewhere (31).

Most *O. anthropi* isolates have been proven to be widely resistant to chloramphenicol and all β -lactams (except imipenem) via production of the AmpC β -lactamase OCH-1. This β -lactamase is chromosomal, inducible, and resistant to inhibition by clavulanic acid (14, 36). Generally, the organism is considered susceptible to gentamicin, fluoroquinolones, sulfamethoxazole-trimethoprim, and colistin (23). The pathogen in the present case was resistant to various β -lactams except carbapenems, gentamicin, ciprofloxacin, levofloxacin, and sulfamethoxazole-trimethoprim. The patient was treated with an oral cephalosporin, to which the pathogen was later shown to be resistant, but he recovered well without any complications, presumably because of the low virulence of the organism. In fact, according to previous studies, immunocompetent patients with *O. anthropi* infection survived and experienced clinical cure without any long-term sequelae (26, 31), and 4 of 5 immunosuppressed patients (organ transplant recipients) with *O. anthropi* bacteremia experienced resolution of bacteremia without antibiotic administration (37).

We performed an environmental survey for *O. anthropi* in the ward to which the patient had been admitted; however, no environmental isolates matched the patient isolates. Therefore, we conclude that the patient had been colonized with the organism somewhere in his body, such as his throat or intestine, and his bacteremia resulted from bacterial translocation across the mucous membrane (38).

Nucleotide sequence accession numbers. Nucleotide sequences of 16S rRNA and *recA* have been deposited in DDBJ/EMBL/GenBank under the accession numbers AB778289 and AB778290.

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We have no conflicts of interest to declare.

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