

Research  
Animal Disease Research—Article

## First Isolation and Characterization of *Ochrobactrum anthropi* from Pig

Shijiang Gu<sup>a,#</sup>, Ruiqing Hou<sup>a,#</sup>, Shengguo Gao<sup>a</sup>, Zhe Sun<sup>a</sup>, Xiangdong Li<sup>a</sup>, Lufeng Zhai<sup>a</sup>, Yunyun Jin<sup>a</sup>, Qiaoyan Zhu<sup>a</sup>, Yonghong Liao<sup>a,\*</sup>, Kegong Tian<sup>a,b,\*</sup>

<sup>a</sup> National Research Center for Veterinary Medicine, Luoyang 471000, China

<sup>b</sup> College of Animal Science and Veterinary Medicine, Henan Agricultural University, Zhengzhou 450002, China



### ARTICLE INFO

#### Article history:

Received 18 March 2019

Revised 31 July 2019

Accepted 2 August 2019

Available online 25 October 2019

#### Keywords:

*Ochrobactrum anthropi*

Pig brain

### ABSTRACT

One Gram-negative *Bacillus* was isolated from a brain sample of a pig with neurological symptoms. Pathological examination showed meningitis at necropsy. *Ochrobactrum anthropi* (*O. anthropi*) was successfully isolated from the brain sample and was confirmed by biochemical reaction results (API 20 NE) and gene sequencing. The strain was highly resistant to  $\beta$ -lactam antibiotics. Mice were experimentally infected with *O. anthropi* and showed typical meningitis. This is the first report on *O. anthropi* isolated from a pig, and indicates that *O. anthropi* may have a broader host spectrum of infection.

© 2020 THE AUTHORS. Published by Elsevier LTD on behalf of Chinese Academy of Engineering and Higher Education Press Limited Company. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

## 1. Introduction

*Ochrobactrum anthropi* (*O. anthropi*) is a Gram-negative *Bacillus* that is tremendously abundant in the environment. It was formerly placed in the category of CDC group Vd [1], which belongs to the family *Brucellaceae* [2]. *O. anthropi* was previously considered to be a human conditional pathogen that mainly infected immunocompromised patients [1,3,4]. However, several reports have indicated that *O. anthropi* can also cause infections in healthy humans [5–7]. The manifestations of *O. anthropi* infections are endophthalmitis [6], endocarditis, meningitis, osteochondritis, osteomyelitis, pancreatic abscess, puncture wound, pelvic abscess, and urinary tract infection [8–14]. Besides human beings, *O. anthropi* has been reported to infect other mammals. Franci et al. [15] were the first to report a healthy canine being infected with *O. anthropi*; this occurred after routine dental cleaning as a result of the anesthetic solution being contaminated with *O. anthropi*. El-Adawy et al. [16] separated *O. anthropi* from the cecum of a turkey. To date, *O. anthropi* has not been reported as occurring in any other species. In this study, we isolated and characterized one strain of *O. anthropi* from a pig for the first time.

## 2. Materials and methods

### 2.1. Isolation of bacteria from pig clinical samples

The pig brain sample was obtained from a commercial farm in Jiangxi Province, China. The pig had neurological symptoms, and the pathological examination showed meningitis at necropsy. Histopathological examination and bacterial separation were carried out from the brain samples. Swabs were taken from the brain sample under aseptic conditions to avoid contamination. The isolate was cultured on tryptone soy agar (TSA; BD Difco™, Becton, Dickinson and Company, USA) containing 10% newborn bovine serum (Zhejiang Tianhang Biotechnology Co., Ltd., China) at 37 °C for 24 h. Gram staining was conducted according to the method described by Gerhardt et al. [17]. The biochemical reaction was profiled using the API 20 NE assay (bioMérieux Inc., France), according to the manufacturer's instruction.

### 2.2. DNA preparation

DNA from bacterial samples cultured for 24 h was extracted by a GENEWIZ Bacteria Genomic DNA Kit (GENEWIZ, China) according to the manufacturer's instruction.

### 2.3. PCR, gene sequencing, and construction of phylogenetic trees

The 16S rRNA gene and *recA* gene were amplified using the methods previously described by Scholz et al. [18]. The forward

\* Corresponding authors.

E-mail addresses: [ebrain04@163.com](mailto:ebrain04@163.com) (Y. Liao), [tiankg@263.net](mailto:tiankg@263.net) (K. Tian).

# These authors contributed equally to this work.

and reverse primers 27f (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492r (5'-AAGTCGTAACAAGGTAACCG-3') were used to generate an approximately 1400 bp fragment of the 16S rRNA gene. The profile of the polymerase chain reaction (PCR) cycle was as follows: 94 °C for 5 min, 30 cycles at 94 °C for 60 s, 56 °C for 30 s, 72 °C for 90 s, and 10 min at 72 °C. The PCR products were analyzed in 1% agarose by gel electrophoresis. The primers for *recA* are *recA-f* (5'-ATGTCTCAAATTCATTGCGAC-3') and *recA-r* (5'-AGCATCTTCTTCCGGTCCGC-3'); these were used to obtain a 1065 bp size band of the *recA* gene. The PCR cycle was as follows: 94 °C for 5 min, 30 cycles at 94 °C for 30 s, 58 °C for 30 s, 72 °C for 60 s, and 10 min at 72 °C. The PCR products were analyzed by electrophoresis in 1% agarose gel.

The PCR products were sent to GENEWIZ for sequencing. The sequences were BLASTed (where BLAST stands for Basic Local Alignment Search Tool) by BLASTnx in the National Center for Biotechnology Information (NCBI) database. CLUSTAL X was used [19] to align the sequences. Phylogenetic trees were built based on the 16S rRNA gene (*rrs*) and the *recA* gene using the neighbor-joining method in Kimura's two-parameter model [20] in MEGA Version 5 [21,22]. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) were shown next to the branches [23].

#### 2.4. Genomic sequencing and BLAST

The genomic sequences were BLASTed by BLAST genomes in the NCBI database. Partial sequences of the *recA* and 16S rRNA and the genomic sequences *Ochrobactrum* and *Brucella* spp. were deposited in GenBank with the accession numbers documented in Table S1 in Appendix A. Supplementary data.

#### 2.5. Antibiotic susceptibility and resistance testing

The isolate was subjected to antibiotic susceptibility and resistance testing by the disk diffusion method presented by Bauer et al. [24]. The isolated strain was cultured on TSA containing 10% newborn bovine serum at 37 °C for 24 h and was then diluted in phosphate-buffered saline (PBS) (0.01 mol·L<sup>-1</sup>, pH 7.2) at 1 × 10<sup>8</sup> CFU·mL<sup>-1</sup> (where CFU stands for colony forming units). The bacterial suspension was spread on the TSA containing 10% newborn bovine serum with a sterile cotton swab, and the paper antibiotic disks (Hangzhou Microbial Reagent Co., Ltd., China) were placed on the inoculated agar surface. The plates were incubated at 35 °C for 24 h prior to determination. The zone diameters of each drug were interpreted using the criteria published by the

Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria from Animals [25].

#### 2.6. Mouse experimental infection study

The isolated strain was cultured on TSA containing 10% newborn bovine serum at 37 °C for 24 h and was then diluted in PBS (0.01 mol·L<sup>-1</sup>, pH 7.2) to obtain a series suspension of about 5 × 10<sup>9</sup>–2 × 10<sup>10</sup> CFU·mL<sup>-1</sup>. BALB/c mice were intraperitoneally injected with the bacterial solution at 1 × 10<sup>9</sup>–4 × 10<sup>9</sup> CFU per mouse with a volume of 0.2 mL per mouse. Histopathological observation was carried out for the mouse brain samples. The animal trial followed the animal protocol approved by the Animal Care and Ethics Committee of the China National Research Center for Veterinary Medicine.

#### 2.7. Histopathological observation

The brain samples were fixed with 10% buffered formalin overnight, followed by embedding in paraffin. Brain tissues were sectioned and stained with hematoxylin and eosin for histopathological evaluation.

### 3. Results

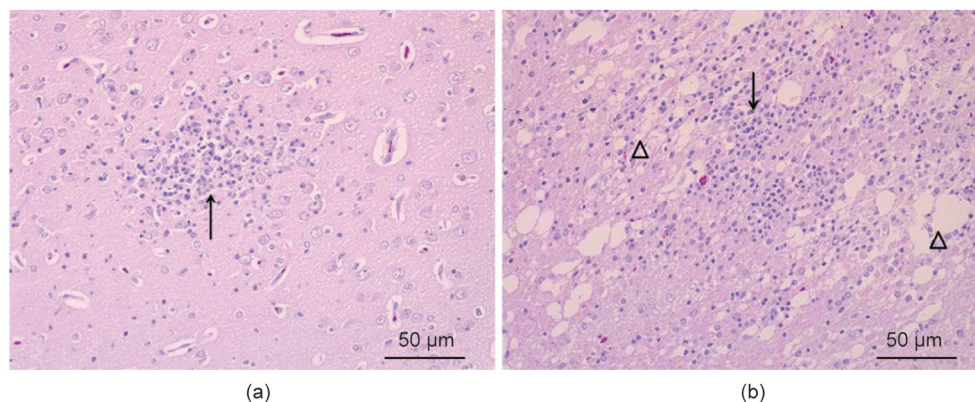
#### 3.1. Histopathological examination and bacterial isolation

The histopathological results for the pig brain sample showed necrotic nodules in the white matter of the brain, necrosis of gray matter neurons, and a large number of neutrophil infiltrations, as shown in Fig. 1. The brain sample was streaked onto TSA plates to obtain pure colonies. The colonies were small and smooth; the cells in the colonies were shown to be Gram-negative bacillus after Gram staining (Fig. 2).

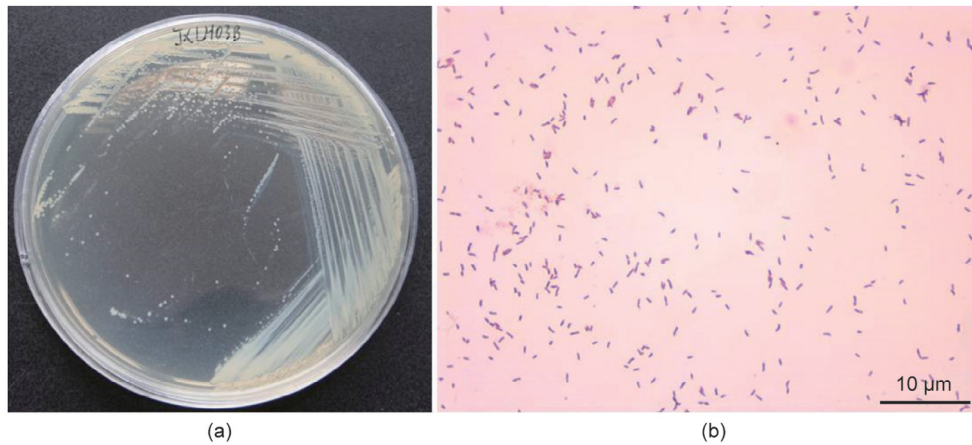
Biochemical identification revealed that the isolated strain was oxidase and catalase positive. The isolate was identified as *O. anthropi* (99.9% probability) by API 20 NE test (Table 1). This isolate was named JXLH03B.

#### 3.2. Sequencing and phylogenetic analysis

The sequence of the JXLH03B 16S rRNA gene was completely identical to that of *Ochrobactrum* sp. (GenBank accession No. MH201346.1), *O. anthropi* (GenBank accession No. LT671861.1), and *Ochrobactrum lupini* (*O. lupini*) (GenBank accession No.



**Fig. 1.** Histopathological observation of the pig brain sample (hematoxylin and eosin staining (H & E), 20×). (a) Necrotic nodules in the white matter of the brain (shown by the arrow); (b) necrosis of gray matter neurons (shown by the triangle) and neutrophil infiltration (shown by the arrow).



**Fig. 2.** Colony morphology and cell morphology of the isolate. (a) Colony morphology of the isolate; (b) microscopic examination of cell morphology (100×) after Gram staining.

**Table 1**

API 20 NE identification of the JXLH03B isolate.

Test name	Reaction/enzyme	Incubation time (h)	Result
NO3	Nitrates reduction		+
TRP	Indole formation		–
GLU	Glucose fermentation		–
ADH	Arginine dihydrolase production		–
URE	Urease production		+
ESC	Hydrolysis ( $\beta$ -glucosidase)		–
GEL	Hydrolysis (protease)		–
PNPG	$\beta$ -galactosidase		–
GLU	Glucose assimilation	24	–
		48	+
ARA	Arabinose assimilation	24	–
		48	+
MNE	Mannose assimilation	24	–
		48	+
MAN	Mannitol assimilation	24	+
		48	+
NAG	<i>N</i> -acetyl-glucosamine assimilation	24	+
		48	+
MAL	Maltose assimilation	24	–
		48	–
GNT	Potassium gluconate assimilation	24	+
		48	+
CAP	Capric acid assimilation	24	–
		48	–
ADI	Adipic acid assimilation	24	–
		48	–
MLT	Malic acid assimilation	24	+
		48	+
CIT	Trisodium citrate assimilation	24	+
		48	+
PAC	Phenylacetic acid assimilation	24	–
		48	–
OX	Cytochrome oxidase	24	+
		48	+
API 20 NE			<i>O. anthropi</i> 99.9%

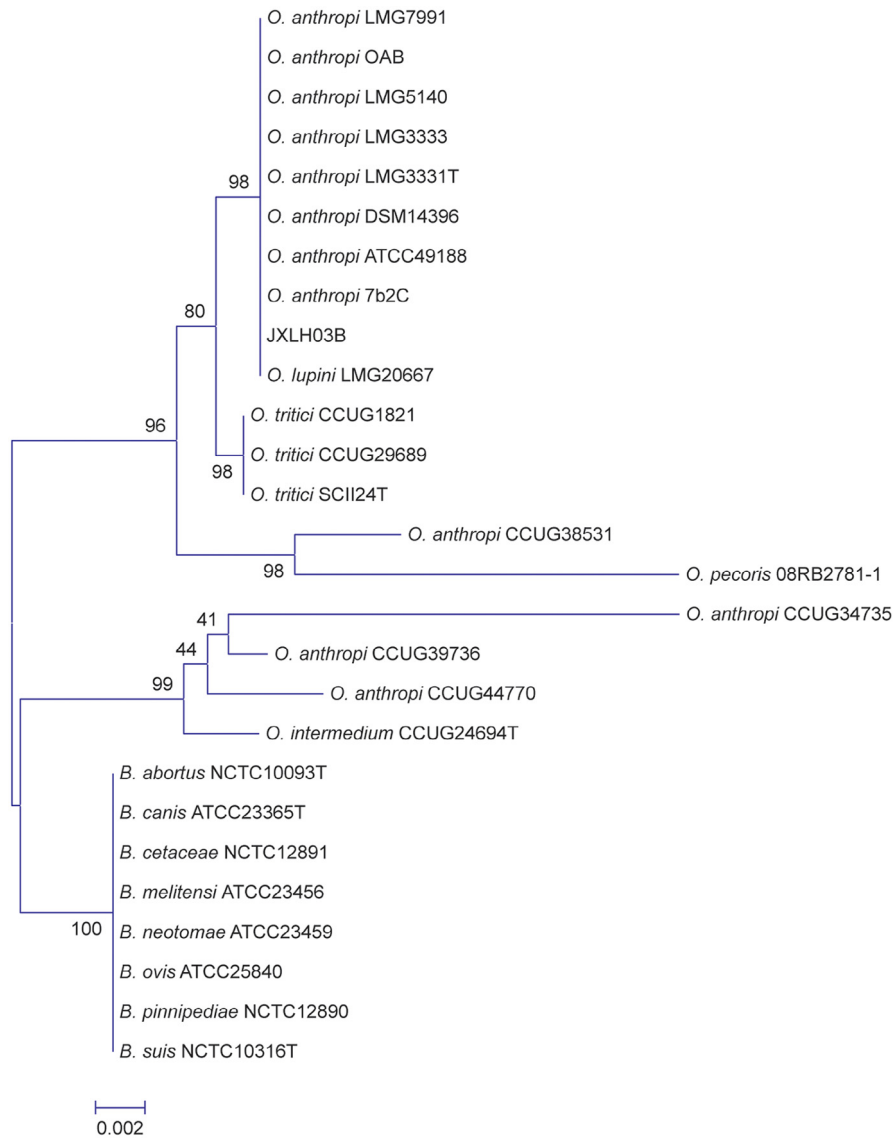
KU217325.1). The *recA* gene was also BLASTed and was shown to be completely identical to *O. anthropi* (GenBank accession No. LT671861.1).

Phylogenetic analysis was performed based on the 16S rRNA gene. As shown in Fig. 3, JXLH03B was grouped with *O. anthropi* LMG 7991, *O. anthropi* OAB, *O. anthropi* LMG 5140, *O. anthropi* LMG 3333, *O. anthropi* LMG 3333T, *O. anthropi* DSM 14396, *O. anthropi* ATCC 49188, *O. anthropi* 7b2C, and *O. lupini* LMG 1821. By contrast,

JXLH03B was grouped with *O. anthropi* DSM 14396, *O. anthropi* LMG 3333, and *O. anthropi* 10CS0094, based on the *recA* gene (Fig. 4).

### 3.3. Genomic sequencing and BLAST

The genomic sequencing results of the JXLH03B isolate were two sequences with a total length of 4 725 913 bp. The G + C content was 56.01%. The lengths of sequence 1 and sequence 2 were



**Fig. 3.** Phylogenetic analysis based on the 16S rRNA gene using the CLUSTREE neighbor-joining method. Scale bar: 0.002 divergent residues per site. The significance of each branch is indicated by a bootstrap value calculated for 1000 subsets.

2 641 072 bp and 2 084 841 bp, respectively. The BLAST results showed that the similarity between the JXLH03B isolate sequences and the two deposited genome sequences of *O. anthropi* was 97.54%–98.08% (Table 2).

### 3.4. Mouse experimental infection study

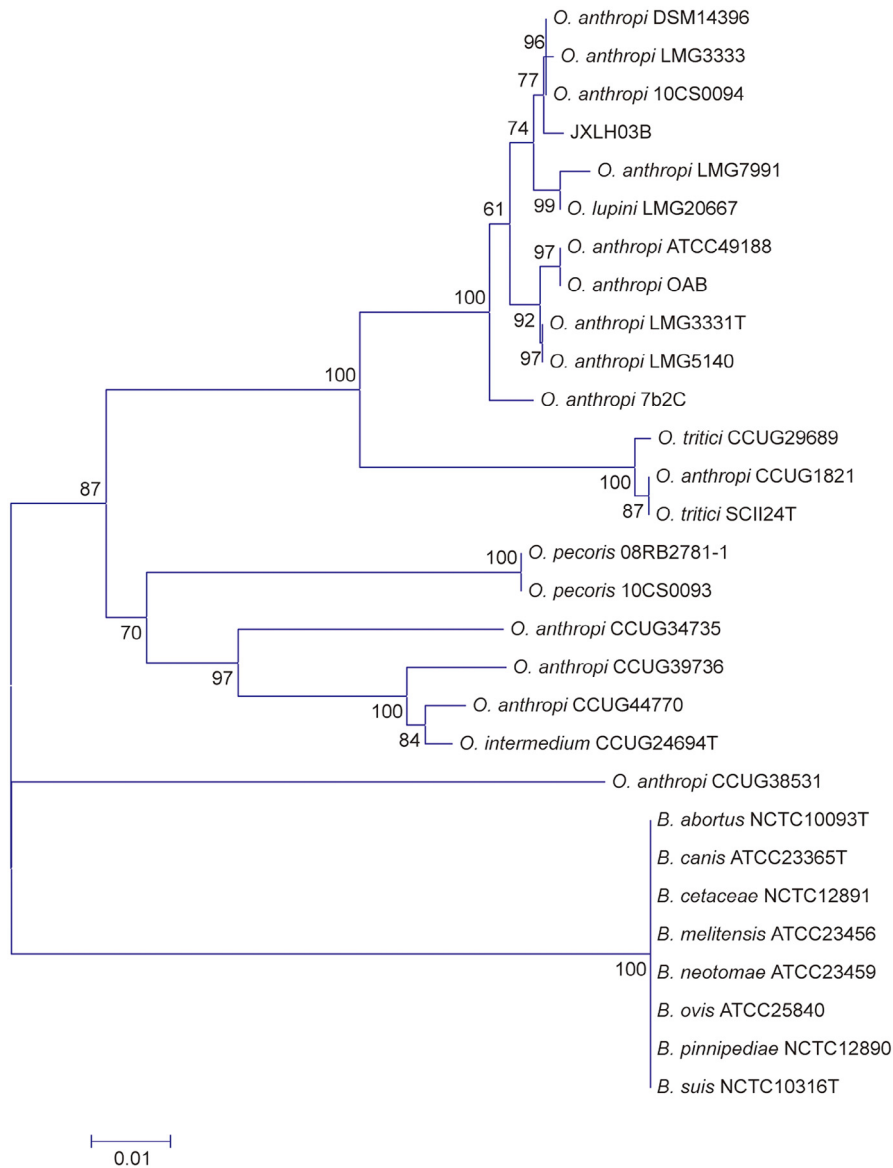
BALB/c mice were infected with JXLH03B at  $1 \times 10^9$ – $4 \times 10^9$  CFU per mouse. Mouse mortality ranged from 0/5 to 5/5 (Table 3). The brains of all the dead mice were sampled for histopathological examination. All of the mice that died after infection had inflammation in the brain tissue. The histopathological results showed several necrotic foci in the brain. The brain parenchyma was liquefied, and a large amount of neutrophils necrosis aggregated to form nodules (shown by the arrow in Fig. 5). Some glial cells were found to collapse (Fig. 5).

### 3.5. Antibiotic sensitivity test

Antimicrobial susceptibility testing was performed using a disk diffusion test following the guidelines of the Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria from Animals [25]. The JXLH03B isolate was susceptible to gentamicin sulfate, amikacin, enrofloxacin, ciprofloxacin, doxycycline, and florfenicol, but was resistant to penicillin sodium, cefotiofur, kanamycin sulfate, lincomycin, and tylosin (Table 4).

## 4. Discussion

*O. anthropi* is an aerobic, non-lactose-fermenting, motile, oxidase-positive, urease-positive, Gram-negative *Bacillus* previously known as “Achromobacter group Vd” [1]. The biochemical reaction profiles (API 20 NE) identified the JXLH03B strain as *O. anthropi*. The genus *Ochrobactrum* belongs to the family



**Fig. 4.** Phylogenetic analysis based on the *recA* gene using the CLUSTREE neighbor-joining method. Scale bar: 0.01 divergent residues per site. The significance of each branch is indicated by a bootstrap value calculated for 1000 subsets.

**Table 2**

Sequence similarity of genomic sequences between JXLH03B and two different *O. anthropi* strains.

<i>O. anthropi</i> strain	Chromosome	GenBank Number	Sequence similarity	
			JXLH03B sequence 1	JXLH03B sequence 2
ATCC49188	Chromosome 1	CP000758.1	98.08%	—
	Chromosome 2	CP000759.1	—	97.82%
OAB	Chromosome 1	CP008820.1	98.08%	—
	Chromosome 2	CP008819.1	—	97.54%

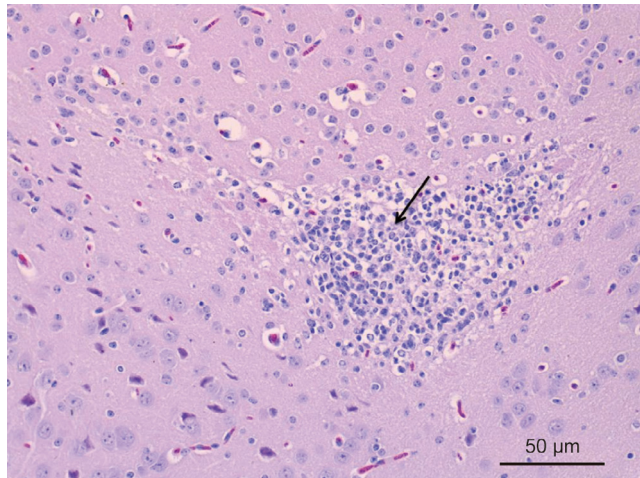
*Brucellaceae* and has a close phylogenetic relatedness to *Brucella* [26]; the genus *Ochrobactrum* comprises about 16 species [27]. 16S rRNA gene analysis was used for the identification and differentiation of *Ochrobactrum* and *Brucella* spp. [2,26,27]. However, this approach—particularly partial sequencing—is prone to misidentifying these pathogens because of their high sequence similarities [27]. Meanwhile, *recA* analysis provides more accurate identification and differentiation [18,27]. We performed the 16S

RNA gene sequencing alignment of the JXLH03B isolate, which could not be completely identified as *O. anthropi*; however, the result of the *recA* gene sequencing comparison identified the JXLH03B isolate as *O. anthropi*.

The infection test in mice showed that the strain had a certain pathogenicity that led to brain lesions. To test the pathogenicity of the JXLH03B strain on pigs, nine 80-day-old healthy pigs were challenged by tracheal injection at doses of  $1.2 \times 10^9$ ,  $1.2 \times 10^{10}$ ,

**Table 3**  
Results from mice infected with JXLH03B.

Infection dose (CFU per mouse)	Mortality
$4 \times 10^9$	5/5
$2 \times 10^9$	3/5
$1 \times 10^9$	0/5



**Fig. 5.** Histopathological observation of the mouse brain sample (H & E, 20 $\times$ ). Necrotic foci occurred in the brain, and neutrophils necrosis aggregated to form nodules (shown by the arrow).

**Table 4**  
Disk diffusion test values and interpretation of the JXLH03B isolate.

Antimicrobial agent	Disk content	Zone diameter (mm)	Interpretation	Interpretive categories and zone diameter breakpoints (mm)		
				R	I	S
Penicillin sodium	10 units	0	R	$\leq 17$	18–20	$\geq 21$
Ceftiofur	30 $\mu$ g	6	R	$\leq 17$	18–20	$\geq 21$
Kanamycin sulfate	30 $\mu$ g	13	R	$\leq 13$	14–17	$\geq 18$
Gentamicin sulfate	10 $\mu$ g	35	S	$\leq 12$	13–14	$\geq 15$
Amikacin	30 $\mu$ g	34	S	$\leq 14$	15–16	$\geq 17$
Enrofloxacin	10 $\mu$ g	34	S	$\leq 12$	13–16	$\geq 17$
Ciprofloxacin	5 $\mu$ g	33	S	$\leq 20$	21–30	$\geq 31$
Lincomycin	2 $\mu$ g	10	R	$\leq 14$	15–20	$\geq 21$
Doxycycline	30 $\mu$ g	45	S	$\leq 10$	11–13	$\geq 14$
Florfenicol	30 $\mu$ g	38	S	$\leq 14$	15–18	$\geq 19$
Tylosin	30 $\mu$ g	10	R	$\leq 15$	16–20	$\geq 21$

S: sensitive, R: resistant, I: intermediate.

and  $1.2 \times 10^{11}$  CFU per pig. However, no animals showed clinical symptoms post-challenge, and no abnormalities were observed in the histopathological examination of the pig brain samples (data not shown). Therefore, we failed to establish the infection model of JXLH03B *O. anthropi* on healthy pigs. In humans, *O. anthropi* mainly infects immunocompromised patients [1,3,4]. We suspect that the *O. anthropi*-infected pig could be immunity compromised due to other pathogen infections. Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) was detected in the diseased pig (data not shown). PRRSV is known to cause immunosuppressive respiratory disease [28]. Therefore, we suspect that the pig was infected with *O. anthropi* due to a compromised immune status. *O. anthropi* is widely distributed in soil and water [2], so we suspect that the pig could have been infected through these pathways.

*O. anthropi* is resistant to practically all  $\beta$ -lactams except carbapenems, partly due to the production of an AmpC  $\beta$ -lactamase [29]. Our results showed that the JXLH03B isolate was resistant to penicillin and ceftiofur, which was consistent with the literature reports [30].

## 5. Conclusion

In this study, we were the first to isolate and characterize *O. anthropi* from a pig. This bacteria was able to experimentally infect mice and led to typical meningitis, which indicated that *O. anthropi* may have a broader host spectrum of infection.

## Acknowledgements

This work is supported by Ten-thousand Talents Program (to Xiangdong Li) and Luoyang Heluo Talent Plan (to Kegong Tian).

## Compliance with ethics guidelines

Shijiang Gu, Ruiqing Hou, Shengguo Gao, Zhe Sun, Xiangdong Li, Lufeng Zhai, Yunyun Jin, Qiaoyan Zhu, Yonghong Liao, and Kegong Tian declare that they have no conflict of interest or financial conflicts to disclose.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.eng.2019.08.014>.

## References

- [1] Holmes B, Popoff M, Kiredjian M, Kersters K. *Ochrobactrum anthropi* gen. nov., sp. nov. from human clinical specimens and previously known as group Vd. Int J Syst Bacteriol 1988;38(4):406–16.
- [2] Lebuhn M, Achouak W, Schlöter M, Berge O, Meier H, Barakat M, et al. Taxonomic characterization of *Ochrobactrum* sp. isolates from soil samples and wheat roots, and description of *Ochrobactrum tritici* sp. nov. and *Ochrobactrum grignonense* sp. nov. Int J Syst Evol Microbiol 2000;50(Pt 6):2207–23.
- [3] Alnor D, Frimodt-Møller N, Espersen F, Frederiksen W. Infections with the unusual human pathogens *Agrobacterium* species and *Ochrobactrum anthropi*. Clin Infect Dis 1994;18(6):914–20.
- [4] Cieslak TJ, Robb ML, Drabick CJ, Fischer GW. Catheter-associated sepsis caused by *Ochrobactrum anthropi*: report of a case and review of related nonfermentative bacteria. Clin Infect Dis 1992;14(4):902–7.
- [5] Berman AJ, Del Priore LV, Fischer CK. Endogenous *Ochrobactrum anthropi* endophthalmitis. Am J Ophthalmol 1997;123(4):560–2.
- [6] Braun M, Jonas JB, Schönherr U, Naumann GO. *Ochrobactrum anthropi* endophthalmitis after uncomplicated cataract surgery. Am J Ophthalmol 1996;122(2):272–3.
- [7] Galanakis E, Bitsori M, Samonis G, Christidou A, Georgiladakis A, Sbyrakis S, et al. *Ochrobactrum anthropi* bacteraemia in immunocompetent children. Scand J Infect Dis 2002;34(11):800–3.
- [8] Romero Gómez MP, Peinado Esteban AM, Sobrino Daza JA, Sáez Nieto JA, Alvarez D, Peña García P. Prosthetic mitral valve endocarditis due to *Ochrobactrum anthropi*: case report. J Clin Microbiol 2004;42(7):3371–3.
- [9] Appelbaum PC, Campbell DB. Pancreatic abscess associated with achromobacter group Vd biovar 1. J Clin Microbiol 1980;12(2):282–3.
- [10] Barson WJ, Cromer BA, Marcon MJ. Puncture wound osteochondritis of the foot caused by CDC group Vd. J Clin Microbiol 1987;25(10):2014–6.
- [11] Chang HJ, Christenson JC, Pavia AT, Bobrin BD, Bland LA, Carson LA, et al. *Ochrobactrum anthropi* meningitis in pediatric pericardial allograft transplant recipients. J Infect Dis 1996;173(3):656–60.
- [12] Christenson JC, Pavia AT, Seskin K, Brockmeyer D, Korgenski EK, Jenkins E, et al. Meningitis due to *Ochrobactrum anthropi*: an emerging nosocomial pathogen. A report of three cases. Pediatr Neurosurg 1997;27(4):218–21.
- [13] Vaidya SA, Citron DM, Fine MB, Murakami G, Goldstein EJ. Pelvic abscess due to *Ochrobactrum intermedium* [corrected] in an immunocompetent host: case report and review of the literature. J Clin Microbiol 2006;44(3):1184–6.
- [14] Jelveh N, Cunha BA. *Ochrobactrum anthropi* bacteremia. Heart Lung 1999;28(2):145–6.
- [15] Franci P, Dotto G, Cattai A, Pasotto D. Lethal septic shock after dental scaling in a healthy dog due to *Ochrobactrum anthropi*-contaminated propofol. J Small Anim Pract 2015;56(5):345–7.

- [16] El-Adawy H, Hotzel H, Tomaso H, Neubauer H, Hafez HM. Isolation and characterization of *Ochrobactrum anthropi* and *Ochrobactrum pecoris* from caecal content of commercial turkeys. *Vet Microbiol* 2012;155(2–4):349–54.
- [17] Gerhardt P, Murray RGE, Wood WA, Krieg NR, editors. *Methods for general and molecular bacteriology*. Washington, DC: American Society for Microbiology; 1994.
- [18] Scholz HC, Tomaso H, Al Dahouk S, Witte A, Schloter M, Kämpfer P, et al. Genotyping of *Ochrobactrum anthropi* by *recA*-based comparative sequence, PCR-RFLP, and 16S rRNA gene analysis. *FEMS Microbiol Lett* 2006;257(1):7–16.
- [19] Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG. The CLUSTAL\_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* 1997;25(24):4876–82.
- [20] Kimura M. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol* 1980;16(2):111–20.
- [21] Saitou N, Nei M. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* 1987;4(4):406–25.
- [22] Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol Biol Evol* 2011;28(10):2731–9.
- [23] Felsenstein J. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 1985;39(4):783–91.
- [24] Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. *Am J Clin Pathol* 1966;45(4):493–6.
- [25] Clinical and Laboratory Standards Institute. Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals: approved standard. 4th ed. Wayne: National Committee for Clinical Laboratory Standards; 2013.
- [26] Velasco J, Romero C, López-Goñi I, Leiva J, Díaz R, Moriyón I. Evaluation of the relatedness of *Brucella* spp. and *Ochrobactrum anthropi* and description of *Ochrobactrum intermedium* sp. nov., a new species with a closer relationship to *Brucella* spp. *Int J Syst Bacteriol* 1998;48(Pt 3):759–68.
- [27] Kämpfer P, Citron DM, Goldstein EJ, Scholz HC. Difficulty in the identification and differentiation of clinically relevant *Ochrobactrum* species. *J Med Microbiol* 2007;56(Pt 11):1571–3.
- [28] Renukaradhya GJ, Alekseev K, Jung K, Fang Y, Saif LJ. Porcine reproductive and respiratory syndrome virus-induced immunosuppression exacerbates the inflammatory response to porcine respiratory coronavirus in pigs. *Viral Immunol* 2010;23(5):457–66.
- [29] Higgins CS, Avison MB, Jamieson L, Simm AM, Bennett PM, Walsh TR. Characterization, cloning and sequence analysis of the inducible *Ochrobactrum anthropi* AmpC beta-lactamase. *J Antimicrob Chemother* 2001;47(6):745–54.
- [30] Oliver JW, Stapenhorst D, Warraich I, Griswold JA. *Ochrobactrum anthropi* and *Delftia acidovorans* to bacteremia in a patient with a gunshot wound. *Infect Dis Clin Pract* 2005;13(2):78–81.