



Case report

Post-surgical meningitis caused by *Klebsiella variicola*

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ABSTRACT

Klebsiella variicola, a member of *K. pneumoniae* phylogroup, can cause severe infectious diseases. We report a case of *K. variicola* meningitis after neurosurgery. The bacterium was isolated from blood and cerebrospinal fluid, and bacterial species identification was carried out by using both matrix-assisted laser-desorption/ionization time-of-flight mass spectroscopy (MALDI-TOF MS) and whole genome sequencing. Initially, the organism was misidentified as *K. pneumoniae* by VITEK® 2; automated system in the clinical laboratory examination. The patient recovered with the combination of surgical drainage and antimicrobial treatment. To our knowledge, this is the first case report of post-surgical meningitis caused by *K. variicola*. As experienced in this case, the automated bacterial identification system popularly being used in the clinical laboratory might not be effective enough for bacterial species identification. The use of MALDI-TOF MS for microbial identification may be helpful to physicians for appropriate management of *K. pneumoniae* phylogroup infection.

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Introduction

Klebsiella variicola is genetically closely related to *K. pneumoniae* and *K. quasipneumoniae*; it is difficult to distinguish the three species using routine biochemical tests [1]. *K. variicola* is an environmental bacterium that colonizes soil and plants [2]. Recently, *K. variicola* has also been reported to cause human infectious diseases; these infections have a higher mortality rate than those caused by *K. pneumoniae* [3]. Because distinguishing *K. variicola* from *K. pneumoniae* is difficult in general clinical laboratories, the clinical importance of *K. variicola* may be underestimated [4]. Therefore, case reports describing the precise identification of *K. variicola* by genome analysis are needed. We report a case of post-neurosurgical meningitis caused by *K. variicola*, in which the causative agent was identified by both mass spectrometry and genome sequencing. To our knowledge, this is the first case report of meningitis caused by *K. variicola*. Furthermore, we reviewed the diagnostic methods used for identifying *K. variicola* infections.

Case report

A 31-year-old woman was transferred to our hospital with sudden onset of severe headache and loss of consciousness. Her medical history was significant for systemic lupus erythematosus on prednisolone 10 mg/day (0.25 mg/kg/day). Based on head computed tomography, she was diagnosed with a subarachnoid hemorrhage. An emergent craniotomy was performed for clipping. Epidural, ventricular, and cisternal drains were inserted during surgery. Thirty-three days after the first surgery, she underwent cranioplasty with; an artificial dura mater inserted, and her own cranial bone, which had been removed for brain decompression during the first surgery, was replaced and fixed with titanium plates. Cefazolin, administered as perioperative prophylaxis, was discontinued on postoperative day one.

Twelve days after the second surgery, she became febrile, and she experienced seizures. Blood and cerebrospinal fluid (CSF) samples were obtained for analysis and culture. On examination, the patient was febrile at 39.3 °C with a pulse rate of 157 beats/min and a Glasgow coma scale of E4VTM2. Examinations of her chest, abdomen, legs, and skin were unremarkable. The following results were observed on CSF analysis: protein level, 814 mg/dL; glucose, undetectable; and total nucleated cell count, 16,000/mm³ (99.6% polynucleated cells). Based on Gram staining of the CSF sample (Fig. 1), we diagnosed bacterial meningitis. Thus, we administered

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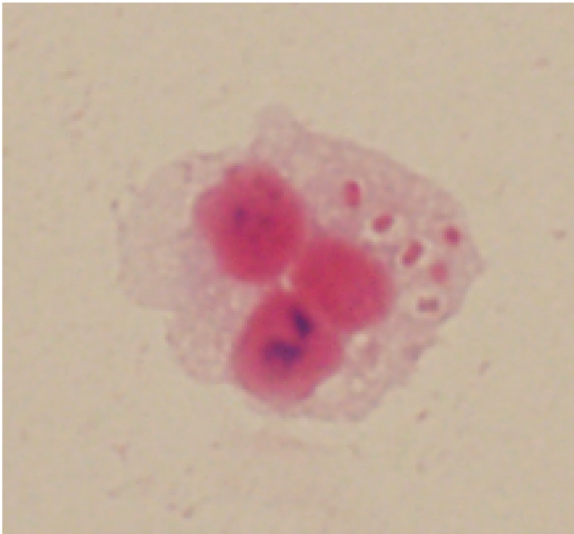


Fig. 1. Gram staining of the cerebrospinal fluid sample showing short gram-negative rods captured by a neutrophil.

intravenous vancomycin 1 g every 12 h and intravenous meropenem 2 g every eight hours as empirical therapy according to the current Infectious Diseases Society of America guidelines [5]. On the following day, she underwent another surgery for open cranial drainage. The artificial dura mater and the replaced cranial bone were removed.

Both blood and CSF cultures were positive. The microorganism isolated from these samples was identified as *K. variicola* using

matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) (MALDI biotyper, Bruker Inc., USA). We also determined the whole genome sequence of the strain detected from the blood sample (JMUB4806), and the sequence was compared with 37 genome sequences, including those of 13 known *K. variicola* strains (Fig. 2). Phylogenetic analysis indicated that JMUB4806 was classified as *K. variicola*. Contrastingly, an automated instrument for infectious disease testing (VITEK®2, bioMérieux S.A., France) identified the causative organism as *K. pneumoniae*.

Based on the antimicrobial susceptibility profile of the detected *K. variicola* strain, therapy was adjusted to intravenous ceftriaxone 2 g every 12 h. Head magnetic resonance imaging (MRI) three days after the third surgery showed severe inflammation in the bilateral frontal lobes and fluid collection in the third ventricle (Fig. 3). The therapy was extended more than four weeks after the third surgery.

Because of the development of drug-induced rash, antimicrobial therapy was transitioned from ceftriaxone to intravenous levofloxacin 500 mg every 24 h and then levofloxacin to intravenous aztreonam 2 g every 8 h. Thirty days after the third surgery, head MRI (Fig. 4) was performed; although hydrocephalus had worsened, the inflammation in the bilateral frontal lobes had diminished, and fluid collection in the third ventricle had disappeared.

Microbiological investigations

The genomic DNA of JMUB4039 was extracted using the DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany). The whole genome was sequenced using the Nextera XT DNA Library



Fig. 2. Phylogenetic relationship of *K. variicola* JMUB4039 with other *Klebsiella* species.

The maximum parsimony tree was constructed using the majority of single nucleotide polymorphisms present in at least 75% of genomes analyzed by kSNP3.0 program (ref. S. N. Gardner et al., 2015 Bioinformatics Vol. 31 2877–2878). The 39 genome sequences analyzed were obtained from seven *Klebsiella* species.

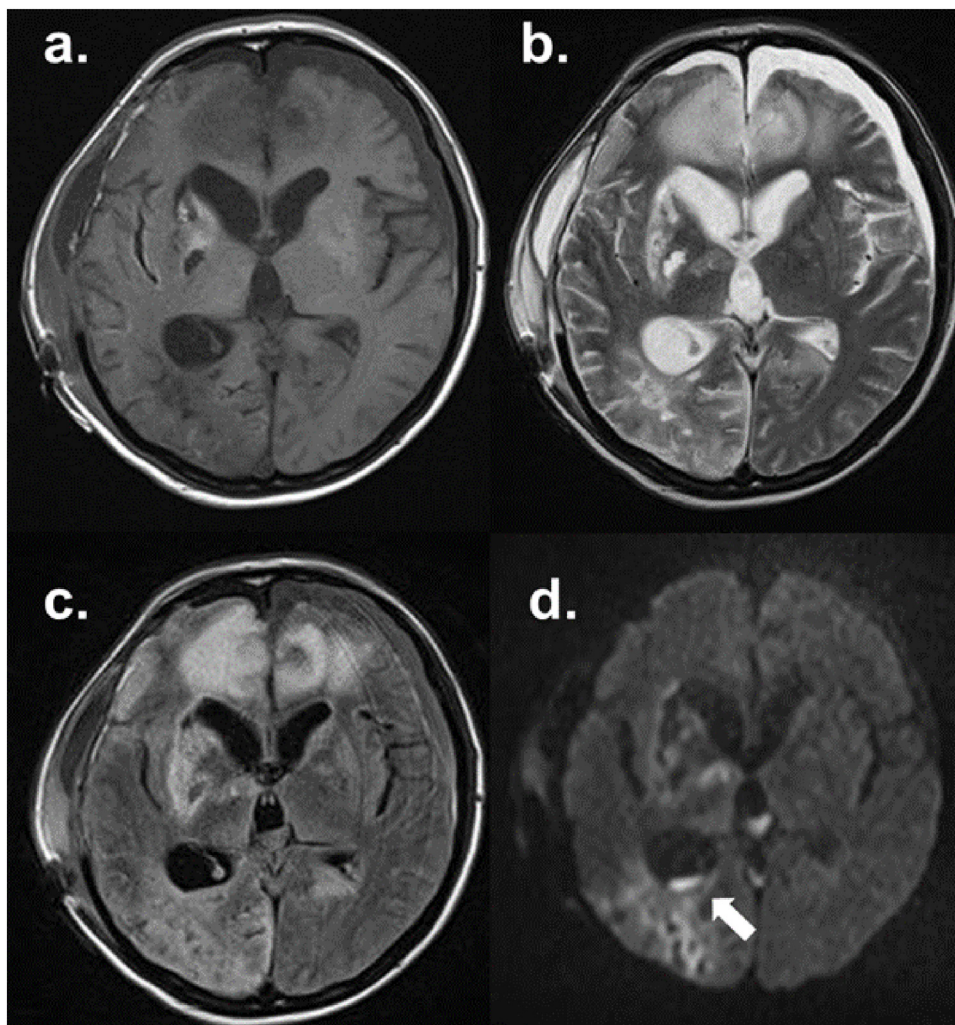


Fig. 3. Head magnetic resonance imaging three days after the third surgery showing severe inflammation in the bilateral frontal lobes and fluid collection in the third ventricle (white arrow).

a. T1, T1-weighted image; b. T2, T2-weighted image; c. FLAIR, fluid-attenuated inversion recovery; d. DWI, diffusion-weighted image.

Preparation Kit and Miseq Reagent Kit v3 (Illumina Inc, USA). The raw reads were assembled by the CLC genomics work bench (CLC bio). The phylogenetic tree was created using kSNP3.0 and FigTree v1.4.3 using JMUB4039 and 38 *Klebsiella* strains whose genome sequences are available on GenBank.

Discussion

We encountered a case of post-surgical meningitis caused by *K. variicola* and treated it successfully. To our knowledge, this is the first case report of meningitis caused by *K. variicola*. In 2004, *K. variicola* was distinguished from *K. pneumoniae* based on the following factors: results of total DNA-DNA hybridization; monophyly observed in phylogenetic analysis that was derived from the sequences of *rpoB*, *gyrA*, *mdh*, *infB*, *phoE*, and *nifH* genes; and distinct phenotypic traits [1]. The draft genome sequence of *K. variicola* was proposed in 2014 [6].

Maatallah et al reported that bacteremic infections caused by *K. variicola* had higher 30-day mortality rates (29.4%) than those of infections caused by *K. pneumoniae* (13.5%) [3]. These results suggested that *K. variicola* has higher virulence than *K. pneumoniae*. Thus, identification of *K. variicola* in clinical settings is important for providing appropriate management and improving

the outcomes of *K. variicola* infections. However, automated instruments used for infectious disease testing might identify *K. variicola* as *K. pneumoniae*. In fact, in our case, organisms detected from blood and CSF cultures were identified as *K. pneumoniae* by VITEK®2, but were identified as *K. variicola* by MALDI-TOF MS and whole genome sequencing. Adonitol fermentation may be useful for distinguishing *K. variicola* (adonitol-negative) from *K. pneumoniae* and *K. quasipneumoniae* (adonitol-positive) [7]. However, the negative predictive value of adonitol fermentation for detecting *K. variicola* is reported to be only 69.8% [7]. Moreover, adonitol fermentation is not routinely performed in clinical laboratories.

In a recently published study, both the sensitivity and specificity of MALDI-TOF MS for detecting *K. variicola* were determined to be 100% [8]. Thus, MALDI-TOF MS may be a suitable method for detecting *K. variicola*. Furthermore, the spread of MALDI-TOF MS among clinical laboratories is expected to facilitate identification of *K. variicola*. In conclusion, to distinguish *K. variicola* from *K. pneumoniae*, clinicians should reanalyze organisms that were identified by automated instruments as *K. pneumoniae*. This could enable physicians to provide appropriate management and improve the outcomes of patients with *K. variicola* infections.

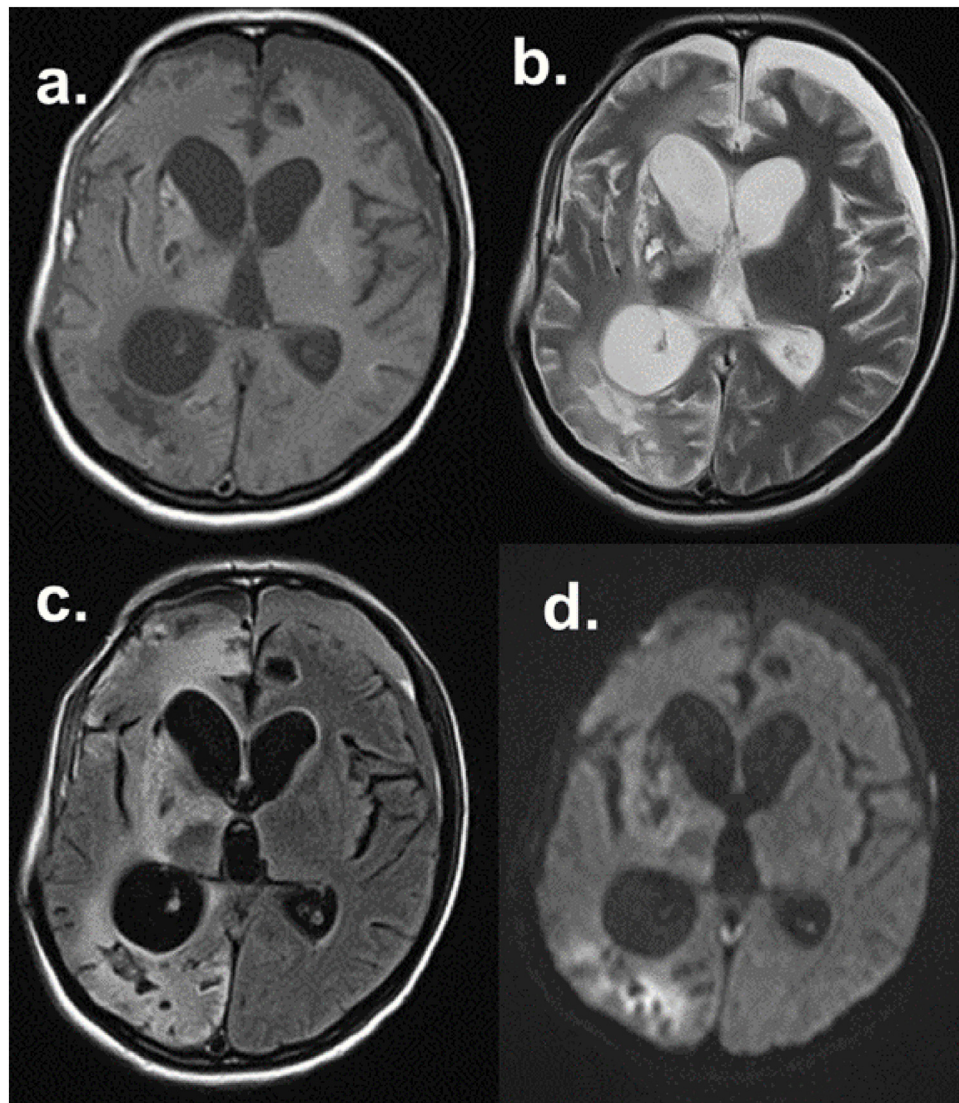


Fig. 4. Head magnetic resonance imaging 30 days after the third surgery showing diminished inflammation in the bilateral frontal lobes and disappearance of fluid collection in the third ventricle.

a. T1, T1-weighted image; b. T2, T2-weighted image; c. FLAIR, fluid-attenuated inversion recovery; d. DWI, diffusion-weighted image.

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Author's contribution

All authors meet the ICMJE authorship criteria.

Dr. Dai Akine, Dr. Teppei Sasahara and Dr. Yuji Morisawa contributed to acquisition, analysis and interpretation of both clinical and microbiological data of the case. Dr. Yohei Ishishita and Dr. Takashi Yamaguchi contributed to analysis and interpretation of clinical data. Dr. Shinya Watanabe and Dr. Longzhu Cui contributed to analysis and interpretation of microbiological data.

All authors revised the work and approved of the final version of the work.

Declaration of Competing Interest

None.

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