Case Report

Fatal sepsis caused by an unusual Klebsiella species that was misidentified by an automated identification system

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This is a description of fatal sepsis caused by infection with Klebsiella variicola, which is an isolate genetically related to Klebsiella pneumoniae. The patient’s condition was incorrectly diagnosed as common sepsis caused by K. pneumoniae, which was identified using an automated identification system, but next-generation sequencing and the non-fermentation of adonitol finally identified the cause of sepsis as K. variicola.

Case report

A 67-year-old woman diagnosed with maxillary sinus cancer in May 2011 was treated with concomitant radiotherapy and cisplatin-based intra-arterial chemotherapy at Osaka University Hospital, Japan. She had not received antibiotics before admission. Physical examination and laboratory findings were unremarkable, and the tumour had decreased in size. However, the maxillary sinus had secreted pus during the course of tumour regression.

After the fourth course of chemotherapy in July 2011, she developed sudden chills and shock. Her consciousness level decreased and 3 L min−1 oxygen was administered, although her chest X-ray did not reveal a shadow. Her leukocyte count and C-reactive protein level were increased.

We suspected sepsis resulting from maxillary sinus bacteraemia. She was admitted to the intensive care unit and was administered meropenem (3 g day−1) and vancomycin (2 g day−1). These are broad-spectrum antibiotics and this combination was empirically considered to be the most effective regimen. The maximum dose of each antibiotic was considered to be appropriate for this patient. Although no pathogenic bacteria were detected in maxillary sinus and respiratory samples, blood samples were collected for bacterial culture. Blood samples were collected in BacT/ALERT FA (aerobic) and FN (anaerobic) bottles (Sysmex/bioMérieux) and were cultured. We found all samples from days 1 (two of two sets), 2 (two of two sets), 3 (one of one set) and 5 (two of two sets) to be positive. Klebsiella pneumoniae, the identification of which was determined using an automated identification system (MicroScan WalkAway; Siemens), was isolated from all blood samples; the results of drug sensitivity tests performed for the bacterium collected from days 1 and 5 showed similar and highly sensitive patterns for most of the antibiotics, including meropenem.

However, the patient’s platelet count decreased, which suggested disseminated intravascular coagulation, and her serum creatinine level increased, which suggested renal failure. She remained in shock although we had administered noradrenaline, and diffuse infiltration shadows appeared in both lung fields on day 5. She died 8 days later from multiple organ failure.

We also performed a metagenomic analysis of 16S rDNA PCR libraries of targeted 280 bp amplicons in the V5–V6 region using total DNA from the patient’s blood as a template and a next-generation DNA sequencer (GS Junior; Roche) as previously described (Nakamura et al., 2011). We found that 85.4% of the 34 706 obtained sequences were identical to Klebsiella variicola (Alves et al., 2006; Rosenblueth et al., 2004). A phylogenetic analysis of the 999 bp rpoB gene sequence amplified by PCR with those of related Klebsiella species was consistent with the sequencing results (Fig. 1). Furthermore, we performed a genetic analysis using cultured colonies, and found that...
sequences were most similar to those of *K. variicola*, as in the blood samples.

We also biochemically analysed the bacterial colonies from the patient, and found that they tested negative for adonitol fermentation, which differentiated *K. variicola* from *K. pneumonaiæ* (Alves et al., 2006; Rosenblueth et al., 2004). In addition, we used the API 50 CHE kit (Sysmex) based on the absence of adonitol in the sugar test and the detected bacteria were identical to the description of *K. variicola* in the API database. We thus concluded that this isolate was *K. variicola* and not *K. pneumonaiæ*.

**Discussion**

The genus *Klebsiella* has increasingly been associated with hospital infections (Rosenblueth et al., 2004). *K. variicola* was initially isolated in Mexico in 2004 based on findings of total DNA–DNA hybridization, monophyly in a phylogenetic analysis derived from *rpoB* gene sequences, and having distinct phenotypic traits. This bacterium does not ferment adonitol and it is mainly found in plants such as bananas, rice, sugar cane and maize (Alves et al., 2006; Rosenblueth et al., 2004).

This report provides clinical evidence of fatal sepsis caused by *K. variicola*. This isolate was initially identified as *K. pneumonaiæ*, based on routine automated identification methods that are popular worldwide (Snyder et al., 2008). *K. variicola* represents <10% of the clinical *Klebsiella* isolates, usually thought to be *K. pneumonaiæ* in humans (Alves et al., 2006; Rosenblueth et al., 2004). The overall agreement between the MicroScan system and the Phoenix automated microbiology system (BD Diagnostics) for clinical isolates of enterobacteria was 97.7% at the genus level and 98.7% at the species level, with 100% (45/45 samples) identity to *K. pneumonaiæ* (Snyder et al., 2008). However, *K. variicola* might be identified as *K. pneumonaiæ* when using routine methods. Our unique finding of this particular *Klebsiella* species may indicate that it has not been identified or recognized before due to problems with the usual automated identification systems. In this case, we used the Siemens system, but the problem might also be present with Sysmex/bioMérieux or BD analysers.

The virulence of *K. variicola* is not clear and severe infection caused by this bacterium has not been described to the best of our knowledge. Our patient died despite the immediate and appropriate administration of antibiotics to which the *K. variicola* isolate was sensitive. Our isolates notably did not express the mucoid phenotype associated with *K. pneumonaiæ* virulence. Although some *K. variicola* strains have candidate virulence genes of the type VI secretion system that can transport virulence factors across bacterial envelopes, the mechanisms of pathogenicity in humans remains unclear (Pukatzki et al., 2009; Sarris et al., 2011).

We detected sequences that were more similar to those of *K. variicola* than *K. pneumonaiæ* using a next-generation sequencer, and subsequently differentiated these *Klebsiella* species by the adonitol test. The results suggested that these tests are valuable when patients present with infection caused by *Klebsiella* species. Although high-throughput next-generation sequencing technologies might be difficult to apply during routine clinical diagnosis, they have potential for clinical genomic studies, as they can vastly exceed the data output of the most sophisticated capillary sequencers based on the Sanger method (Pareek et al., 2011). We have performed metagenomic sequencing with blood cultures to increase detection rates and confirm pathogens in severe cases of suspected bacteraemia/sepsis. Detection and genetic analysis of pathogens directly from blood samples could be performed within a day, although routine blood cultures take a few days to detect pathogens. This time saving would also benefit patients. We suggest the development of routine genetic analysis of blood samples along with cultures.

In conclusion, we described fatal sepsis caused by *K. variicola*, which automated tests usually identify as *K.
pneumoniae. There appears to be a need for a system that can readily distinguish *K. variicola* from *K. pneumoniae* in clinical microbiology laboratories, and the pathogenicity of *K. variicola* should be fully investigated.

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**References**


