

An Investigation into Laboratory Misidentification of a Bloodstream *Klebsiella variicola* Infection

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Bloodstream infections represent a major burden on the United States health care system, with the number of cases of severe sepsis exceeding 750,000 per year (1) and representing 10% of all intensive care unit (ICU) admissions (2). It has also been shown that patient survival rates directly correlate with the ability to identify these infections early, with a 7.6% increase in the mortality rate occurring for every hour treatment is delayed (3). To improve upon the reported time to identification of bloodstream infections in our laboratory, we implemented the use of the automated Verigene system (Nanosphere Inc., Northbrook, IL). The Verigene system is a multiplex, automated microarray-based nucleic acid diagnostic test for the identification of genus, species, and genetic resistance determinants from a broad panel of the most common Gram-negative and Gram-positive organisms isolated from blood cultures. Here we describe two bloodstream isolates that were reported as “not detected” when using the Verigene system but were subsequently identified as *Klebsiella pneumoniae* by both the Vitek 2XL identification/antimicrobial susceptibility testing and Vitek MS matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) systems (both from bioMérieux, Marcy l’Etoile, France). *K. pneumoniae* is included in the Verigene system Gram-negative blood culture panel, prompting us to investigate a potential false-negative result by the Verigene system. Our inquiry revealed that these isolates were actually another *Klebsiella* species, *Klebsiella variicola*.

A 27-year-old female with a history of systematic lupus erythematosus complicated by lupus nephritis presented to the medical ICU for acute hypoxic respiratory failure requiring intubation, attributed to a drug interaction. This patient’s condition was further complicated by hematuria, kidney failure, pancytopenia, hemolytic anemia, oral thrush, and gastrointestinal bleeding from cytomegalovirus colitis. Multiple sets of blood cultures were drawn throughout the patient’s hospital stay. Bottles from two blood culture sets drawn 3 weeks apart were flagged positive on the Bactec FX (Becton Dickinson, Sparks, MD) blood culture instrument and were found to be growing Gram-negative rods. First, the aerobic (BD Bactec Plus Aerobic/F culture vial) and anaerobic (BD Bactec Anaerobic/F culture vial) bottles from a peripheral blood draw were flagged positive, followed by the anaerobic bottle only from an arterial line blood draw.

The Verigene system was utilized as our first-line assay for rapid organism identification in positive blood cultures, but no organism was detected. Subsequently, the isolates grew on blood and MacConkey agar, followed by identification through utilization of Vitek 2XL and Vitek MS. Both instruments identified each of the isolates as *K. pneumoniae* with a 97% probability score from Vitek 2XL. Since *K. pneumoniae* is an organism included in the Verigene system Gram-negative blood culture panel, these isolates should have been identified if present in the positive blood cul-

TABLE 1 Primers used to amplify KVAR_0717 (*yggE*)^a

Amplification target	Amplicon size (bp)	Primer direction	Primer sequence
<i>Klebsiella</i> spp.	330	Forward	AGAATGCTTTTCCCGTTCTGA
		Forward	AGAATGCTTTTGCCGTTCTGA
		Forward	AGAATGCTTTTACCGTTCTGA
		Forward	AGGATGCTTTTGCCATTCTGA
		Reverse	TGGAGAAACGACAGTGAAGTT

^a The primers shown were used to amplify the KVAR_0717 gene of *Klebsiella* spp. in order to use this amplified sequence for genetic sequencing.

tures. Therefore, we began our investigation of what appeared to be two false-negative results.

Bioinformatic and biochemical analyses were utilized to further characterize these two isolates. Our goal was to determine whether these isolates were indeed *K. pneumoniae*, as reported by the two Vitek instruments, or another closely related member of the genus *Klebsiella* that would not be identified by the Verigene system. In 2012, a Japanese group reported the human pathogenicity of *K. variicola*, a species with close genetic similarity to *K. pneumoniae* (4), prompting us to examine whether our isolates could be this species. Since >95% of *K. variicola* isolates have been shown not to ferment the carbohydrate adonitol (5) (whereas *K. pneumoniae* ferments this carbohydrate), we performed a biochemical analysis using Vitek 2XL to analyze adonitol fermentation. Neither isolate fermented adonitol, which supported our notion that these isolates were *K. variicola*. Next, a genome comparison study of *K. pneumoniae* and *K. variicola* identified KVAR_0717 (*yggE*) as a *de novo* target on the basis of sequence differences and a bioinformatic analysis was performed by amplification of this gene using *Klebsiella* species-specific primers (performed at ACGT Inc., Wheeling, IL) (Table 1), followed by nucleotide sequencing on an ABI 3730XL genetic analyzer to definitively identify both *Klebsiella* isolates to the species level (performed at Nanosphere Inc., Northbrook, IL). Bioinformatic analysis identified the two isolates as *K. variicola* with >99.5% confidence. On the basis of sequencing, a phylogenetic tree was generated that demonstrates the relatedness of these two isolates

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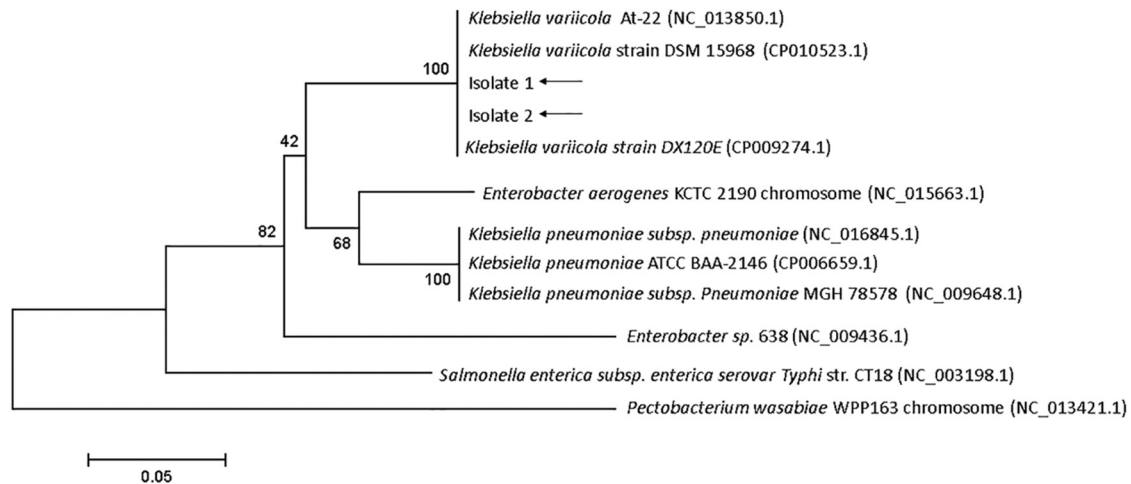


FIG 1 The phylogeny of clinical isolates 1 and 2 was determined by the neighbor-joining method from partial sequences of KVAR_017 (*yggE*) and was prepared with MEGA6 software. The percentages of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) are shown next to the branches. All reference sequences were obtained from NCBI, and NCBI reference sequence numbers are included.

to other known bacterial sequences (Fig. 1) and clearly shows that these two isolates cluster with *K. variicola*.

Our investigation revealed that both Vitek 2XL and Vitek MS misidentified the isolates in question as *K. pneumoniae*, when further analysis revealed that they were both actually *K. variicola*. Vitek 2XL uses sugar fermentation reactions to biochemically determine bacterial identity, and Vitek MS uses the profile of bacterial proteins to determine identity. In contrast, the Verigene system, which is a microarray-based nucleic acid diagnostic test and includes *K. pneumoniae* in its identification panel, did not misidentify the isolates and correctly reported them as not identified. Since *K. variicola* is closely related to *K. pneumoniae*, it is difficult to distinguish between the two species by commonly used methods such as 16S rRNA gene sequencing (6, 7). The Verigene system is able to distinguish between the two species because the *K. pneumoniae* probes used in the assay have a low enough sequence identity with *K. variicola* (88% versus 100% for *K. pneumoniae*) to select against *K. variicola* as a target. *K. variicola* was first isolated from plants (6) and has previously been identified as a cause of fatal sepsis (4). Isolates of *K. variicola* have previously been shown to have lower antibiotic resistance rates than other *Klebsiella* species (5). A recent study by Maatallah et al. also found lower antibiotic resistance rates but linked *K. variicola* bloodstream infections to a higher mortality rate than infections caused by *K. pneumoniae*. Also, no additional known virulence factors in *K. variicola* were identified that could explain the increased risk of death (8). These findings demonstrate that lower antibiotic resistance rates do not necessarily correlate with better treatment outcomes in *K. variicola* infections. With this in mind, proper identification of *K. variicola* will be critical in the further evaluation of its clinical significance as an underreported infectious agent and in the development of more tailored *K. variicola* treatment options. Since both Vitek 2XL and Vitek MS are widely used for isolate identification in clinical labs, our data reveal one way in which *K. variicola* infections may be currently underestimated.

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