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The Brief Case: *Klebsiella variicola*—Identifying the Misidentified

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CASE

A 72-year-old Japanese male with a history of colorectal cancer with metastases to the liver and peritoneum presented to the emergency department with fever and abdominal pain and was subsequently found to have Gram-negative bacteremia. The patient had undergone numerous chemotherapy treatment courses since his diagnosis 18 months prior as well as a central hepatectomy with hepaticojejunostomy. One year after the resection, he developed recurrent ascending cholangitis from a biliary stricture with an associated bile leak, necessitating the placement of a biliary stent and percutaneous drain. Despite drain placement, he presented to the hospital several times with Gram-negative bacteremia, most frequently growing either *Escherichia coli* or *Klebsiella pneumoniae*. Prior to transfer of his care to our institution 1 month prior, he had been placed on rotating prophylactic antibiotics, including amoxicillin-clavulanic acid, ciprofloxacin, and trimethoprim-sulfamethoxazole.

Vital signs on admission revealed a fever of 103.0° F, heart rate of 100 bpm, and a blood pressure of 96/64 mm Hg. On exam he was cachectic, lethargic, and diaphoretic. His respiratory exam was notable for rales in the left lower base, and he had right-upper-quadrant abdominal pain with deep palpation but did not have rebound tenderness. His biliary drain contained scant amounts of dark green bilious fluid, and the rest of his exam was unremarkable.

The initial complete metabolic panel (CMP) revealed an elevated alkaline phosphatase of 763 U/liter, which was above his baseline of 200 to 300 U/liter. His white blood cell (WBC) count was 6.99×10^3 cells/mm^3. A paracentesis was performed which was inconsistent with spontaneous bacterial peritonitis, noting only 11/mm^3 neutrophils. A computed tomography (CT) scan of his abdomen and pelvis with contrast revealed that the percutaneous biliary drain terminated in the hepaticojejunostomy and that the intrahepatic bile duct remained undrained, raising concern for possible obstruction.

Two sets of bioMérieux BacT/Alert FN Anaerobic Plus and FA Aerobic Plus blood cultures were obtained. One of the two sets grew Gram-negative bacilli that could not be identified by our lab's Verigene Gram-negative blood culture panel. The sample was next tested using a Biofire Filmarray blood culture identification panel, which returned *K. pneumoniae*. The blood cultures were sent to our reference lab, Kaiser Permanente Northwest Regional Laboratory, which identified the pathogen as *K. pneumoniae* using a Vitek mass spectrometry matrix-assisted laser desorption/ionization time of flight (MALDI-TOF MS) instrument with version 2.0 software. An adonitol fermentation test was also performed on a Vitek 2 instrument running version 7.01 software, which was negative. These discordant results suggested that this could be a *Klebsiella* species other than *K. pneumoniae*. Our institution's probe for *K. pneumoniae* used with the Verigene panel does not cross-react with *Klebsiella variicola* and therefore returned the result "no molecular ID detected." The Vitek MS MALDI-TOF software did not have *K.*

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For answers to the self-assessment questions and take-home points, see https://doi.org/10.1128/JCM.00825-18 in this issue.

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variicola in the database and the isolate was misidentified as *K. pneumoniae*. The microbiology laboratory personnel suggested that we explore the possibility that our isolate was *K. variicola* based on a recent report by Berry et al. on the misidentification of *K. pneumoniae* (1).

Susceptibility testing revealed that the isolate was resistant to amoxicillin/clavulanic acid, cefazolin, ceftriaxone, ceftazidime, ciprofloxacin, gentamicin, and piperacillin-tazobactam and susceptible to amikacin, cefepime, tobramycin, and trimethoprim-sulfamethoxazole. Subsequent blood cultures were negative. Since the isolate had a relatively resistant antibiotic profile, we attempted further species identification. DNA was extracted from the culture, and the KVAR_0717 gene of the *Klebsiella* species was PCR amplified and then subjected to Sanger sequencing. This gene is a target based on sequencing differences between *K. variicola* and *K. pneumoniae* (1). Based on sequence homology determined using the Basic Local Alignment Search Tool (BLAST) (https://blast.ncbi.nlm.nih.gov/Blast.cgi), the isolate was identified as *K. variicola*. The patient had his biliary drain repositioned and was ultimately discharged on cefepime for 2 weeks.

DISCUSSION

Conventional blood cultures can take 2 to 3 days to produce results, which has driven the development of advanced bacterial identification technologies that can decrease wait time by 24 to 36 h for a microbiologic diagnosis. These methods, however, are not infallible, and reporting misidentifications is imperative to the improvement of molecular diagnostic tools. Systems like Biofire, Vitek 2, Verigene, and MALDI-TOF MS can correctly identify Gram-negative organisms accurately a vast majority of the time, which is particularly helpful when resistance markers are detected. However, a percentage of isolates are still misidentified. Databases used in common molecular diagnostics platforms such as Verigene, Vitek, or MALDI-TOF MS remain incomplete, particularly with respect to rare bacterial species.

K. variicola is a nitrogen-fixing bacterium that was first identified in 2004 based on phylogenetic analysis of the *rpoB* gene sequence and DNA-DNA hybridization. It is estimated that 2.5% to 10% of *K. pneumoniae* isolates are actual misidentifications of *K. variicola* (1–4). *K. variicola* is likely under-recognized because of its similar phylogenetic and biochemical properties to those of *K. pneumoniae*. Until recently *K. variicola* and *K. quasipneumoniae* have not been included in common molecular databases, so they either were not identified or were misidentified. It is important to understand the limitations of molecular diagnostics and that correct identification is dependent on the databases available.

Misidentifications have been propagated over time because of the inclusion of misidentified *K. variicola* and *K. quasipneumoniae* sequences in American Type Culture Collection (ATCC) public gene sequence databases and in research studies. The inclusion of these gene sequences has conflated *K. pneumoniae* with *K. variicola* and *K. quasipneumoniae* until recently. The current Bruker MALDI-TOF MS microorganism database still does not include *K. quasipneumoniae*, leading to continued misidentification (4). *K. variicola* was recently added to the Bruker reference library, but prior to 2015 the reference library contained at least one strain of *K. variicola* that was misassigned as a *K. pneumoniae* strain (4).

Historically, *K. pneumoniae* was separated into three different phylogenetic groups based on sequencing of *gyrA* and *parC* genes. KpI is the largest cluster which includes *K. pneumoniae* subspecies; KPII and KPIII clusters include *K. quasipneumoniae* and *K. variicola*, respectively (2, 5, 6). KPIII strains can be adonitol positive and have similar *gyrA* sequences.

It was once thought that adonitol testing could differentiate these clusters, but this has been disproven (2). The inability to ferment adonitol can be a feature of *K. variicola*, but there can be variation in this test. Brisse et al. found that adonitol was positive in 4.3% of the KPIII isolates and Alves et al. showed that *K. variicola* was adonitol positive in about 20% *of K. variicola* isolates (6). Although biochemical techniques can be

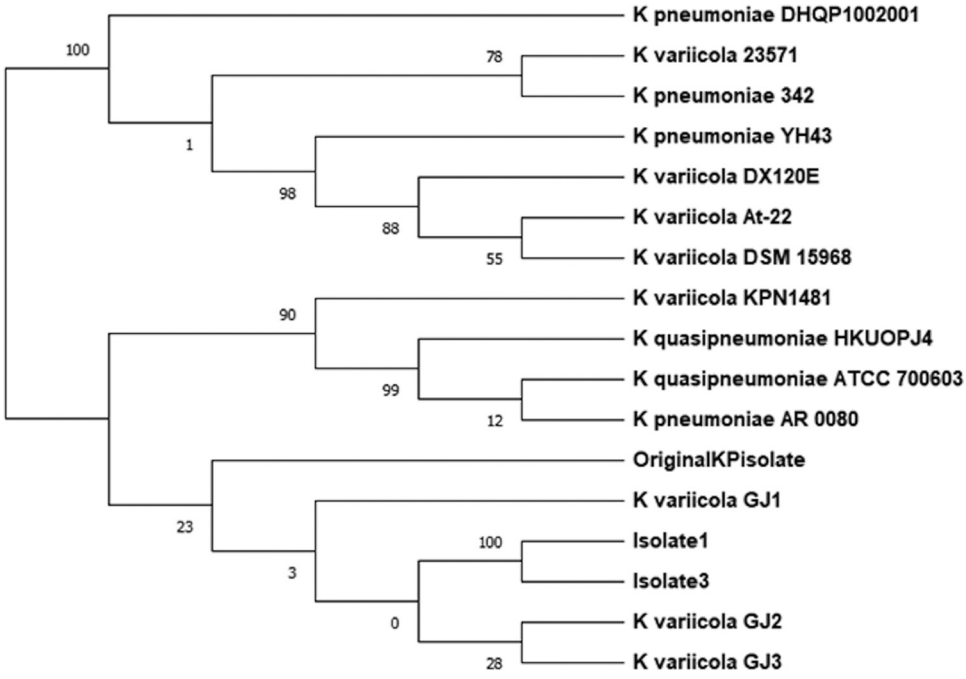

FIG 1 The evolutionary relationship was inferred by using the maximum likelihood method using the general time-reversible model. The tree with the highest log likelihood is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. The isolates reported in the paper are OriginalKPisolate, isolate 1, and isolate 3.

suggestive, these tests are not absolute given the overlap that can occur (6, 7). At this time the only definitive method to differentiate species is by whole-genome sequencing (WGS) or targeted sequencing.

Once we identified our original isolate as *K. variicola*, which was a first for our institution, we investigated other *K. pneumoniae* blood isolates that may have been misidentified. In 2017, there were a total of 56 *K. pneumoniae* blood isolates at our institution. Of the 56, there were 6, excluding the original isolate, that were not identified by Verigene but were later identified as *K. pneumoniae* on a Vitek MALDI-TOF MS. We were able to perform direct PCR and genetic sequencing on four of these isolates. Two of the four were identified as *K. variicola*, and the other two were more closely related to *K. quasipneumoniae*. In Fig. 1 all three *K. variicola* isolates are presented in the phylogenetic tree.

Overall, the genetic distances between all the *Klebsiella* strains were very close but not identical. For our three *K. variicola* strains, the first seven matches in the BLAST database had identity scores of >97%. The top matches included *K. variicola* strains At-22, DSM 15968, DX 120E, and GJ1-GJ3. There is a well-defined group with isolates 1 and 3, based on a high bootstrap value but not a value as high as that among the other clusters.

Based on the phylogenetic analysis, our *K. variicola* strains share the same ancestor as *K. variicola* strains GJ1, GJ2, and GJ3, which are carbapenem-resistant strains found to possess the $bla_{\text{NDM-9}}$ gene (Fig. 1) though, notably, our isolate did not contain a carbapenemase. *K. variicola* strains GJ1, GJ3, and GJ3 have previously been identified as environmental strains isolated from the Gwangju tributary in South Korea (8). Genomic sequencing of these isolates revealed that they carry the $bla_{\text{NDM-9}}$ gene, a new variant of the New Delhi metallo-beta-lactamase gene, within the IncFII(y) plasmid. In addition to carbapenems, these isolates were also resistant to beta-lactams, sulfonamides, trimethoprim, aminoglycoside, fosfomycin, and fluoroquinolones (8). The IncFII-type plasmid has also been involved with spreading the $bla_{\text{ctx-M-15}}$ gene among *Escherichia coli* strains (8). Other case reports have found *K. variicola* strains that produce *K.*

pneumoniae carbapenemase (KPC) and New Delhi metallo-beta-lactamase 1 (NMD-1) genes (4). In this case series, due to an incomplete database, 2% of ESBL-producing *K. pneumoniae* isolates were misidentifications of *K. variicola* and *K. quasipneumoniae*, and 12.6% non-ESBL-producing *K. pneumoniae* isolates were misidentifications of *K. quasipneumoniae* (4). Our original isolate had a resistance profile similar to profiles of the strains from South Korea except that it was not carbapenem resistant. The other two *K. variicola* isolates from our institution had more favorable resistance patterns.

While management of this case was not affected by the misidentification, correct species identification carries important prognostic and epidemiologic implications. The importance of correctly identifying strains is emphasized by a study done in Sweden, which found an increased mortality in patients infected with *K. variicola* compared to that in patients infected with *K. pneumoniae* isolates although virulence factors could not be identified (9). Though this study revealed a statistically significant difference in the mortality rates between the KPI and KPIII clades, the multivariate analysis was limited due to the small number of events that met the primary endpoint (30-day mortality).

In attempts to rectify the issue of misidentification, Garza-Ramos et al. developed a multiplex PCR to aid in correctly identifying *K. variicola*. They found *K. variicola* had a prevalence of 2.1% but that 56% of the isolates were multidrug resistant (3). This multiplex PCR provides the ability to differentiate between *K. variicola* and *K. pneumoniae*, which would be helpful in epidemiological and population studies. We did not use this multiplex PCR technique in our study and did not compare our method with the method of Garza-Ramos et al. as it was beyond the scope of our project, but we recognize that a more comprehensive approach such as whole-genome sequencing would provide greater genetic resolution of our isolate.

Continued efforts to understand *K. variicola* phylogenetic and genomic properties are needed to aid in understanding the prevalence, virulence, and resistance patterns of *K. variicola*. Misidentification can have significant clinical implications, especially given the unique resistance profiles of each organism. As new species are identified by DNA sequencing, molecular databases can be updated to facilitate more accurate identification. Updating the databases would allow us to understand an organism's epidemiology and the role that this could potentially play in the emergence of multidrug-resistant organisms, which may also affect the antibiotics reported on susceptibility panels. Further studies are needed to assess how these updates will impact actual clinical management.

SELF-ASSESSMENT QUESTIONS

1. Which antibiotic-resistant gene found on the IncFII(y) plasmid was associated with the *Klebsiella variicola* isolate from the Gwangju tributary?
    a. *K. pneumoniae* carbapenemase (KPC)
    b. Cefotaxime-M beta-lactamase (CTX-M)
    c. Imipenem 1 (IMP-1)
    d. Variant of the NDM gene ($bla_{\text{NDM-9}}$)
2. What two genes can be sequenced in the performance of phylogenetic analysis to aid in dividing *Klebsiella* species into different clusters?
    a. *rpoB* and *gyrA*
    b. *parC* and *rpoB*
    c. OXA-1 and *rpoB*
    d. *gyrA* and *parC*
3. Which of the following choices can definitively differentiate *Klebsiella variicola* from *Klebsiella pneumoniae* at the species level?
    a. Adonitol fermentation
    b. Nitrogen fixation
    c. Whole-genome sequencing
    d. Indole positivity

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