

Frequency of *Klebsiella variicola* and *Klebsiella quasipneumoniae* in Clinical Isolates: Antimicrobial Resistance Profiles

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The *Klebsiella pneumoniae* species complex (KpSC) comprises opportunistic Gram-negative bacteria responsible for both hospital-acquired and community-acquired infections. Although *K. pneumoniae* is the most prevalent species, *K. variicola* and *K. quasipneumoniae* are also associated with severe infections. Their accurate identification is limited by the phenotypic methods routinely used in clinical laboratories, which fail to discriminate among these species. This limitation is particularly concerning given their ability to acquire and disseminate antimicrobial resistance genes, thereby complicating the treatment and control of such infections, as well as hindering epidemiological surveillance of the less frequently identified species. Therefore, this study aimed to determine the frequency of *Klebsiella variicola* and *Klebsiella quasipneumoniae* within the *Klebsiella pneumoniae* species complex among clinical isolates, and to characterize their antimicrobial resistance profiles. To achieve this, a total of 241 bacterial isolates initially identified as *Klebsiella pneumoniae* by conventional automated phenotypic methods were included. Species-level identification within the *K. pneumoniae* species complex (*K. pneumoniae*, *K. variicola*, and *K. quasipneumoniae*) was performed using multiplex PCR. Ten isolates (*K. variicola*, n=5; *K. quasipneumoniae*, n=5) were further selected for whole-genome sequencing using Oxford Nanopore technology. Genome assembly was conducted with Flye, and antimicrobial resistance genes were identified using Kleborate and Abricate, in combination with the ResFinder, CARD, and NCBI AMRFinderPlus databases. Overall, 88% (n=212) of the isolates were identified as *K. pneumoniae*, 6.6% (n=16) as *K. quasipneumoniae*, and 5.4% (n=13) as *K. variicola*. Most isolates originated from urine (34%, n=83) and blood (30%, n=73) samples. In addition, more than 50% of the isolates exhibited resistance to carbapenems, while 38% of *K. variicola* isolates were resistant to ceftazidime-avibactam. Whole-genome sequencing further revealed substantial genetic diversity and a predominance of multidrug-resistant profiles. Specifically, most *K. quasipneumoniae* isolates exhibited limited resistance associated mainly with chromosomal beta-lactamases and porin alterations, although some carried carbapenemases such as KPC-2 together with additional resistance determinants. In contrast, *K. variicola* isolates displayed extensive resistomes, including multiple carbapenemases and beta-lactamases, as well as determinants associated with resistance to quinolones, aminoglycosides, and other antimicrobial classes. Collectively, these findings demonstrate the underrecognized diversity within the KpSC and the frequent misidentification of non-*pneumoniae* species in clinical laboratories. Despite their lower prevalence, *K. variicola* and *K. quasipneumoniae* exhibited heterogeneous resistance profiles, including multidrug-resistant phenotypes and carbapenemase production, underscoring their clinical relevance. Taken together, these results highlight that accurate species-level identification and genomic characterization are essential to strengthen epidemiological surveillance and optimize antimicrobial therapy within the KpSC.

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