

CLINICAL IMAGE

Sputum Gram stain of hypervirulent *Klebsiella pneumoniae*

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The patient was an 82-year-old woman with a history of nodular bronchiectatic *Mycobacterium avium* complex pulmonary disease and had been taking erythromycin for several years. She was hospitalized for 3 months because of pneumothorax and subsequent rehabilitation. One week after discharge, she visited the emergency department with a 2-day history of fever and dyspnea. Her oxygen saturation was 93% with 3L/min oxygen via a nasal cannula. Chest radiography and computed tomography revealed extensive infiltrative shadows in both lungs, and ampicillin-sulbactam was initiated to treat bacterial pneumonia. Whole-body computed tomography revealed no disseminated infectious lesions other than pneumonia. Gram stain of the sputum revealed large Gram-negative rods (GNRs) surrounded by a clear area ("halo"; [Figure 1](#)), and blood culture yielded positive results for GNRs. Consequently, the antibiotic was altered to meropenem. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry identified the organism as *Klebsiella pneumoniae*, which produced extended-spectrum beta-lactamase. Our strain produced a viscous rope with a length of >5 mm, and the string test was considered to be positive. Meropenem was switched to cefmetazole based on the results of susceptibility testing. After 2 weeks of antibiotic therapy, her condition improved, and the patient was discharged. Genetic analysis of the serotype and virulence genes was performed using a previously described method.¹ The serotype of the isolated *K. pneumoniae* was K2, and it was positive for virulence genes, including *rpmA*, *rpmA2*, *iroN* and *iutA*, suggesting that our isolate was hypervirulent *K. pneumoniae*.²

Gram stain is a rapid and useful tool for identifying the causative organism of bacterial pneumonia, and a large GNR is highly specific

for *K. pneumoniae*.³ All strains of *K. pneumoniae* produce extracellular polysaccharide capsules,² and it is widely recognized that in Gram stain, *K. pneumoniae* has a transparent surrounding area ("halo") which indicate the presence of a capsule. However, no available data exist regarding the proportion of *K. pneumoniae* isolates in which thick capsules can be visualized through Gram stain.

Capsule overproduction is the essential pathogenicity of hypervirulent *K. pneumoniae*.² Through capsule stain and transmission electron microscopy, it was observed that the capsule of

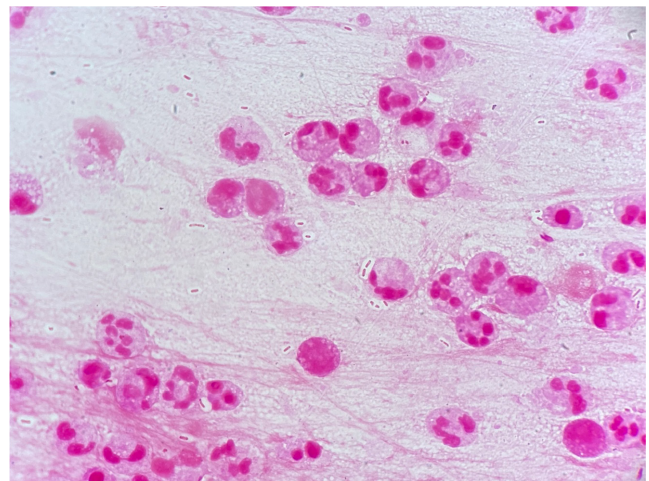


FIGURE 1 Large-sized Gram-negative rods in sputum detected after Gram staining (×1000, oil immersion field). Bacteria are surrounded by a thick transparent area ("halo").

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hypervirulent *K. pneumoniae* with *rmpA* and *rmpA2* genes, which are relevant to capsule overproduction, was considerably thicker than that of the hypovirulent strain without such virulence genes.⁴ Taken together, observation of a large GNR with a thick capsule may not only indicate that it is *K. pneumoniae* but also that it is a highly virulent strain. The Gram stain is a useful test that has been employed in clinical microbiology for more than 100 years, and it may still yield new insights by reevaluating it in conjunction with advances in molecular studies.

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CONFLICT OF INTEREST STATEMENT

The authors have stated explicitly that there are no conflicts of interest in connection with this article.

ETHICS STATEMENT

Our patient consented in writing to the publication of the case, and we believe that is sufficient for ethical considerations.

PATIENT CONSENT STATEMENT

Written informed consent was obtained from the patient to publish this article.

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