Answer to Photo Quiz: *Klebsiella pneumoniae* Bacteremia Showing Filamentous Forms and Spheroplasts Due to the Presence of Subinhibitory Concentrations of β -Lactams

(See page 2475 in this issue [[doi:10.1128/JCM.01523-12](https://doi.org/10.1128/JCM.01523-12)] for photo quiz case presentation.)

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The morphological features are consistent with filamentous forms and spheroplasts of Gram-negative bacilli produced in the presence of β -lactam antimicrobials (1). The Gram stain of the blood culture medium showed elongated Gram-negative microbial forms approximately 1 μ m in diameter and up to several hundred micrometers long. Additionally, spherical enlargements, 5 to 8 μ m in diameter, were seen along some of the bacterial forms (see Fig. 1a in the Photo Quiz). The organisms fluoresced orange under UV illumination when stained with acridine orange, with a morphology similar to that observed on the Gram stain (see Fig. 1b in the Photo Quiz), while no staining was detected on calcofluor white-stained preparations. This interpretation was confirmed the following day by isolation of Gram-negative bacilli, which were identified as *Klebsiella pneumoniae* isolates resistant to ampicillin and susceptible to all other agents tested, including piperacillin-tazobactam.

The peptidoglycan layer of the bacterial cell wall maintains the shape of the organism and prevents lysis that would result from high intracellular osmotic pressure (2). Peptidoglycan synthesis is catalyzed by various transpeptidases or penicillin-binding proteins (PBPs). The β -lactams act by binding to PBPs and can kill by three different mechanisms: rapid lysis, production of spherical cells, or filamentation (3). Many β -lactams kill bacteria by two or all three of these mechanisms at concentrations achieved in serum, while subinhibitory concentrations typically result in formation of elongated or spherical forms. During replication of rod-shaped bacteria, a central, transverse septum is formed and split to form two daughter cells, followed by elongation of the peptidoglycan layer. Septation is mediated by the transpeptidase PBP 3, and

cells become elongated if this enzyme is absent or inhibited. Elongation is mediated by the transpeptidase PBP 2, and cells become enlarged and spherical in the absence or inhibition of this enzyme. In contrast, rapid lysis occurs when PBP 1A and PBP 1B are inhibited. Piperacillin has particularly high affinity for PBP 3 (4), and subinhibitory concentrations therefore result in formation of elongated cells, as was found in the case presented here. Clinicians and microbiologists should be aware of the morphological changes in Gram-negative organisms associated with subinhibitory concentrations of β -lactams.

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