AMERICAN SOCIETY FOR MICROBIOLOGY | Journal of Clinical Microbiology®

PHOTO QUIZ

Answer to Photo Quiz: *Streptococcus pneumoniae*

Cinzia Tavano,[a,b] Renée Pasciuta,[a] Silvia Carletti,[a] Cristina M. Ossi,[a] Rossella Ieri,[a] Paola Cichero,[a] Nicola Clementi,[b] Roberto Burioni,[b] Massimo Clementi,[a,b] Nicasio Mancini[a,b]

[a]Laboratory of Microbiology and Virology, San Raffaele Scientific Institute, Milan, Italy
[b]Department of Microbiology and Virology, University "Vita-Salute" San Raffaele, Milan, Italy

KEYWORDS *Streptococcus pneumoniae*, blood culture, diagnostics, sepsis

The figure in the photo quiz shows an uncommon Gram stain of a *Streptococcus pneumoniae* isolate. The subcultured isolate did not grow either on blood agar (BBL Columbia agar with 5% sheep blood; Becton Dickinson) or on chocolate agar (chocolate agar-PolyViteX; bioMérieux) even after 72 h of incubation under aerobic conditions (37°C; 5% CO_2). No other media were subcultured from the positive aerobic bottles.

Several factors may reduce the vitality of bacteria, thus modifying their appearance on the Gram stain and their ability to grow. In *Streptococcus pneumoniae*, one of these factors is certainly autolysis (1). Autolysis plays a role not only in reducing *in vitro* growth but also in pneumococcal pathogenesis, thereby shielding bacteria from the immune system and increasing toxin release (1). Autolysis is induced by specific enzymes (autolysins), which are activated in the stationary phase and degrade cell wall peptidoglycan, therefore modifying the cell appearance on the Gram stain (2).

After 30 h of incubation, a single anaerobic blood culture bottle turned positive, and bacteria with the same Gram stain features were evidenced. Only the use of anaerobically incubated Schaedler agar plates (BBL Schaedler agar with vitamin K1 and 5% sheep blood; Becton Dickinson) facilitated the growth of the isolate, and its definitive identification as *Streptococcus pneumoniae* was through matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) (Vitek MS; bioMérieux). To further elicit the growth, the cultured blood was inoculated in brain heart infusion broth (BBL brain heart infusion; Becton Dickinson). After a further 72 h, a significant growth was obtained on subcultured Schaedler agar plates, allowing antimicrobial susceptibility testing to be performed.

The isolate was susceptible to the most commonly used antipneumococcal antibiotics, including benzylpenicillin (MIC $\leq$ 0.06 μg/ml) (Vitek 2; bioMérieux), ceftriaxone (MIC $\leq$ 0.12 μg/ml), erythromycin (MIC $\leq$ 0.12 μg/ml), and levofloxacin (MIC, 0.5 μg/ml). Empirical therapy was therefore shifted to treatment with ceftriaxone only for a total of 14 days, leading to her making a complete recovery.

Importantly, during the complete investigation period, urinary pneumococcal antigen (UPA) (Alere BinaxNOW *Streptococcus pneumoniae*) test results were repeatedly and persistently negative. The sensitivity of this test varies from 59% to 75% in different studies (3, 4), and false-negative reports have been associated with some clinical factors, including low C-reactive protein (CRP) levels and warfarin therapy (5). A recent report described a low sensitivity to serotypes not included in the then-available seven-valent conjugated vaccine formulation (6). Interestingly, our isolate was later recognized as belonging to serotype 3, a serotype not included in the seven-valent vaccine.

This case can be seen as an example of the challenging laboratory presentation of pneumococcal pneumonia and sepsis. It is important to remember that in clinically suggestive cases, the suspicion of pneumococcal etiology should be borne in mind

Citation Tavano C, Pasciuta R, Carletti S, Ossi CM, Ieri R, Cichero P, Clementi N, Burioni R, Clementi M, Mancini N. 2019. Answer to Photo Quiz: *Streptococcus pneumoniae*. J Clin Microbiol 57:e00175-18. https://doi.org/10.1128/JCM.00175-18.

Editor Paul Bourbeau

Address correspondence to Cinzia Tavano, tavano.cinzia@hsr.it.

See https://doi.org/10.1128/JCM.00160-18 in this issue for photo quiz case presentation.

Published 30 January 2019

even when difficult-to-grow diplococci with an apparent negative Gram stain result are visualized and even where UPA test results are persistently negative.

REFERENCES

1. Martner A, Skovbjerg S, Paton JC, Wold AE. 2009. *Streptococcus pneumoniae* autolysis prevents phagocytosis and production of phagocyte-activating cytokines. Infect Immun 77:3826–3837. https://doi.org/10.1128/IAI.00290-09.
2. Mellroth P, Daniels R, Eberhardt A, Rönnlund D, Blom H, Widengren J, Normark S, Henriques-Normark B. 2012. LytA, major autolysin of *Streptococcus pneumoniae*, requires access to nascent peptidoglycan. J Biol Chem 287:11018–11029. https://doi.org/10.1074/jbc.M111.318584.
3. Laijen W, Snijders D, Boersma WG. 2017. Pneumococcal urinary antigen test: diagnostic yield and impact on antibiotic treatment. Clin Respir J 11:999–1005. https://doi.org/10.1111/crj.12453.
4. Zalacain R, Capelastegui A, Ruiz LA, Bilbao A, Gomez A, Uranga A, España PP. 2014. *Streptococcus pneumoniae* antigen in urine: diagnostic usefulness and impact on outcome of bacteraemic pneumococcal pneumonia in a large series of adult patients. Respirology 19:936–943. https://doi.org/10.1111/resp.12341.
5. Watanabe H, Uruma T, Tazaki G, Kikuchi R, Tsuji T, Itoh M. 2015. Clinical factors associated with negative urinary antigen tests implemented for the diagnosis of community-acquired pneumococcal pneumonia in adult patients. Med Princ Pract 24:189–194. https://doi.org/10.1159/000369931.
6. Shoji H, Domenech A, Simonetti AF, González A, García D, Cubero M, Martí S, Maeda M, Tubau F, Liñares J, Domínguez MA, Carratalà J, Ardanuy C. 2018. The Alere BinaxNOW pneumococcal urinary antigen test: diagnostic sensitivity for adult pneumococcal pneumonia and relationship to specific serotypes. J Clin Microbiol 56:e00787-17. https://doi.org/10.1128/JCM.00787-17.