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Journal Title: Open Forum Infectious Diseases
Volume: Volume 3, Number 4
Publisher: Oxford University Press (OUP) | 2016-01-01, Pages ofw206-ofw206
Type of Work: Article | Final Publisher PDF
Publisher DOI: 10.1093/ofid/ofw206
Permanent URL: <https://pid.emory.edu/ark:/25593/rtd8r>

Final published version: <http://dx.doi.org/10.1093/ofid/ofw206>

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Accessed November 20, 2019 12:21 PM EST

Gram-Positive Diplococci in a Cerebrospinal Fluid Gram Stain

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A 5-month-old girl presented with meningitis after receiving amoxicillin for bilateral otitis media. The cerebrospinal fluid (CSF) Gram stain suggested *Streptococcus pneumoniae*: Gram-positive diplococci with a surrounding clear area indicative of a bacterial capsule. Her CSF and blood cultures grew penicillin-resistant *S pneumoniae* serotype 35B. This serotype is not included in the 13-valent pneumococcal conjugate vaccine (PCV-13) and has been identified as a cause of invasive pneumococcal disease in the post-PCV-13 era.

Keywords. Gram stain; meningitis; *Streptococcus pneumoniae*.

A 5-month-old previously healthy female presented with fever and new-onset seizures. Four days before presenting to medical attention, she developed a temperature up to 38.6°C, cough, nasal congestion, and vomiting. She had been evaluated at an urgent care center at that time and was diagnosed with bilateral otitis media. She was prescribed amoxicillin. She continued to have fever and presented with respiratory failure and a prolonged seizure. Her physical exam was notable for meningismus. A computed tomography scan of the head noted bilateral middle ear and mastoid opacification. Analysis of the patient's cerebrospinal fluid (CSF) revealed a leukocyte count of 478 cells/μL (69% polymorphonuclear cells), a glucose concentration of 4 mg/dL, and a protein concentration of 213 mg/dL. A cytopsin-prepared CSF Gram stain revealed Gram-positive cocci arranged in pairs, some of which were within leukocytes (Figure 1). A clear area ("halo") was apparent around the intraleukocytic organisms, suggesting an encapsulated bacterial species. The patient's blood and CSF cultures grew *Streptococcus pneumoniae* serotype 35B, a capsular serotype not included in the 13-valent pneumococcal conjugate vaccine. Notably, the isolates exhibited a penicillin minimum inhibitory concentration value of 2 μg/mL, which is considered resistant when interpreted using the parenteral penicillin meningitis breakpoints for *S pneumoniae* [1].

Marked declines have occurred in invasive pneumococcal serotypes included in or related to the 13-valent pneumococcal conjugate vaccine (PCV-13) in both children and adults (through herd protection) in the United States. Previously uncommon serotypes are now responsible for an increasing proportion of invasive disease, and serotype 35B was the fourth most common invasive isolate (8%) among United States children under 5 years of age in 2012–2013 [2]. Overall, antibiotic-resistant invasive pneumococcal cases declined significantly among young children by 3 years after PCV-13 licensure [2]. In a study of invasive and noninvasive pneumococcal isolates from across the United States in 2012–2013, serotype 35B had become the most common penicillin-nonsusceptible serotype (24.8%)

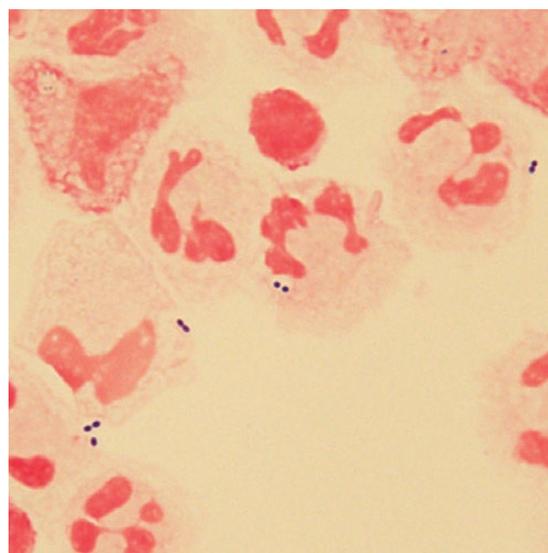


Figure 1. Cytopsin-prepared Gram stain of the patient's cerebrospinal fluid (original magnification, ×1000).

Received 27 August 2016; accepted 22 September 2016.

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Open Forum Infectious Diseases®

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DOI: 10.1093/ofid/ofw206

and the fourth most common multidrug-resistant serotype (6.4%) [3].

Cells of *S pneumoniae* are elongated (“lancet-shaped”) cocci that are surrounded by a polysaccharide capsule and predominantly organized in pairs (diplococci). Other Gram-positive organisms that cause meningitis in neonates and infants include *Streptococcus agalactiae* (Group B *Streptococcus*), a coccus ordered in pairs and chains, and *Listeria monocytogenes*, a short rod.

Acknowledgments

We acknowledge the clinical microbiology laboratory technicians at Children’s Healthcare of Atlanta for their technical expertise, the Georgia Emerging Infections Program, and the US Centers for Disease Control and Prevention for serotyping the isolate.

Potential conflicts of interest: E. J. A. reports personal fees for consulting from AbbVie and clinical trial funding and editorial assistance from MedImmune, outside the submitted work.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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