A Rapidly Fatal Infection With Haemophilus influenzae Serotype E Harboring bla<sub>ROB-1</sub>: The Dilemma of Safe De-escalation in the Setting of Potential Extended-Spectrum β-Lactamase Production

John Hanna, Yetunde Ogunesan, Erik Snesrud, Rosalyn Maybank, Ana Ong, Yoon Kwak, Anthony Jones, Patrick McGann, and Emil Lesho

Walter Reed Army Institute of Research, Silver Spring, Maryland

DOI: 10.1093/ofid/ofy302

This work is written by (a) US Government employee(s) and is in the public domain in the US. Published by Oxford University Press on behalf of Infectious Diseases Society of America 2018.

CASE

A 66-year-old female with asthma-chronic obstructive lung disease overlap syndrome (ACOS) and rheumatoid arthritis (RA) presented with respiratory distress, altered mental status, and sepsis. Her family reported no sick contacts, recent travel, pets, or other epidemiological exposures, and she had been feeling well, with no fevers, chills, or respiratory symptoms. Her RA did not require treatment with biologic response modifiers, prednisone, or methotrexate, and there was no evidence of pulmonary involvement. She was not continuously oxygen dependent, but she was using 2L intermittently immediately before admission. In the preceding 5 years, she visited the emergency department once and was admitted once for her ACOS.

Upon admission, she required urgent endotracheal intubation and was empirically treated with ampicillin, cefepime, vancomycin, azithromycin, and oseltamivir. Her Charlson Comorbidity Index (CCI), Pitt Bacteremia (PBS), and Simplified Acute Physiology Scores were 4, 3, and 77, respectively. Chest x-ray showed consolidation of her right lung. Gram-stain of her sputum revealed pleomorphic Gram-negative coccobacilli. A multiplexed polymerase chain reaction respiratory viral panel was negative. Blood and sputum cultures grew nonhemolytic, slightly opaque, dome-shaped colonies on chocolate agar. However, there was no growth on sheep's blood or MacConkey agars. Over the next 48 hours, her temperature and PBS rose to 108.7°F and 8, respectively. She developed shock and multiorgan failure. The organism was identified as a β-lactamase-producing Haemophilus influenzae (HI), and de-escalation to ampicillin-sulbactam, or ceftriaxone, or piperacillin-tazobactam was debated. Due to the fulminant nature of her presentation, the possibility of higher-level resistance to β-lactams, and the fear of possibly inducing further extended spectrum β-lactamase resistance by using ceftriaxone [1, 2], cefepime was continued until susceptibilities could be confirmed. Four days after admission, numerous septic brain emboli occurred, and she died before susceptibility information became available.

Using microbroth dilution and/or disk diffusion tests prepared according to Clinical Laboratory Standards Institute methods and interpretative breakpoints, the isolate was resistant to penicillin and ampicillin, intermediate to amoxicillin-clavulanate and piperacillin-tazobactam, and susceptible to azithromycin, cefepime, and ceftriaxone. Phylogenetic analysis and antibiotic resistance gene content, based on its whole genome sequence (WGS), revealed that the isolate was serotype E and sequence type 966. It was most closely related to isolates from the Netherlands and Italy, but it was distinct from a large outbreak at a military base in Fort Benning, Georgia (Figure 1). The isolate contained bla<sub>ROB-1</sub>, a β-lactamase-encoding gene, and 66 putative virulence-associated genes. Amino acid mutations S130G and R244K, which have been shown to confer resistance to some extended spectrum cephalosporins and β-lactamase inhibitors [1], were also present.

DISCUSSION

We describe an interesting isolate and a noteworthy clinical outcome. The isolate is notable for its resistance and virulence gene content, phenotypic susceptibility, and the challenges encountered during antibiotic susceptibility testing (AST). The patient's course is remarkable for its fulminance in someone not substantially immunocompromised, nor at the extremes of age. Indeed, her initial CCI between 3 and 4 predicted a 10-year survival of 50%–70%. However, before the special test media could be acquired by the hospital laboratory, the patient had died. This emphasizes the value of having local antibiograms available to guide empiric treatment. In addition, having the AST for the specific isolate available would have fostered stewardship by informing us that it would have been safe to de-escalate to ceftriaxone. Given the presence of β-lactamase production, and a gene with mutations that encode resistance...
to extended-spectrum β-lactams and some β-lactamase inhibitors, we were extremely hesitant to de-escalate. Although HI infections have increased 50% in some US locations over the last decade [3], and atypical presentations such as pyogenic infections of the genitourinary system have emerged [4], descriptions of type E strains using comparative genomics, and the dilemmas HI can present to clinicians and nonreference laboratories, remain scarce.

Although matrix-assisted laser desorption ionization time-of-flight (MALDI-ToF) has simplified the identification of HI, AST remains complicated [2]. Most hospital laboratories do not provide AST results on individual isolates or antibiograms for HI due to its fastidious nature and the specialized testing media required. In addition, variation of inoculum concentration may lead to falsely resistant results with some β-lactams, particularly for β-lactamase-producing strains such as the one in this report [5]. Furthermore, no interpretative breakpoints exist for many relevant antibiotics. In fact, one of the major AST agencies is only now establishing breakpoints for piperacillin-tazobactam. The other major AST agency states that “results of susceptibility testing using breakpoints provided for oral β-lactams, macrolides, and ketolide agents may not be useful for the management of individual patients” [2, 5]. Even with advances such as MALDI-ToF and WGS, susceptibility testing and preliminary results still can vex clinical decision making. Clinical laboratories continue to struggle generating timely and actionable AST reports, due to regulatory and technical barriers [6, 7]. In addition, although WGS has become the “standard-of-care” for epidemiology, evidence for using WGS-inferred AST to guide clinical decision making is lacking [8].

For HI, susceptibility to most β-lactams is usually predicted by susceptibility to ampicillin [2]. Ampicillin resistance often occurs via β-lactamases production or altered penicillin-binding proteins (PBPs). Strains with altered PBPs are referred to as β-lactamase-negative, ampicillin resistant (BLNAR), but AST agencies debate what constitutes a BLNAR strain and whether BLNAR strains should also be considered resistant to amoxicillin/clavulanate, ampicillin/sulbactam, cefaclor, cefetamet, cefprozil, cefuroxime, loracarbef, and piperacillin/tazobactam despite apparent in vitro susceptibility to these agents [2, 5].

Of the 2 main β-lactamase-encoding genes in HI, *bla*<sub>TEM</sub> and *bla*<sub>ROB-1</sub>, the former is far more prevalent, with only approximately 10% of strains worldwide harboring *bla*<sub>ROB-1</sub> [1, 2]. Both genes are plasmid-mediated and encode class A serine enzymes that are typically inhibited by clavulanate and tazobactam [1]. ROB-1 degrades cefprozil but not the oxyimino-cephalosporins, ceftriaxone, and cefotaxime. Cefaclor exposure may select for *bla*<sub>ROB-1</sub>-carrying strains, because approximately 70% of cefaclor-resistant strains harbor *bla*<sub>ROB-1</sub>. *bla*<sub>ROB-1</sub> is more prevalent in the United States and Mexico, where cefaclor use is relatively high [1, 2]. The above suggests that piperacillin-tazobactam, ampicillin-sulbactam, or ceftriaxone are reasonable empiric choices; for BLNAR strains, cefepime should be considered [2].

**CONCLUSIONS**

Finally, this report demonstrates the ongoing need for further investigations of bacterial virulence correlates and mechanisms [9, 10]. Toward that end, we make the isolate and its WGS available to other researchers.
Acknowledgments

Disclaimer. The views expressed in the manuscript are solely those of the authors and are not to be construed as official or representing those of the Department of Defense.

Potential conflicts of interest. All authors: No reported conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest.

References
1. Galán JC, Morosini MI, Baquero MR, et al. Haemophilus influenzae bla(ROB-1) mutations in hypermutagenic deltamC Escherichia coli conferring resistance to cefotaxime and beta-lactamase inhibitors and increased susceptibility to cefaclor. Antimicrob Agents Chemother 2003; 47:2551–7.
2. Tristram S, Jacobs MR, Appelbaum PC. Antimicrobial resistance in Haemophilus influenzae. Clin Microbiol Rev 2007; 20:368–89.
3. New York State Department of Health. Communicable diseases in New York State: cases reported in 2016. Available at: https://www.health.ny.gov/statistics/diseases/communicable/2016/docs/select.pdf. Accessed 15 September 2018.
4. Howard-Anderson J, Satola SW, Collins MH. Breech at the border: an atypical case of invasive Haemophilus influenzae in a patient on a novel immunotherapeutic. Open Forum Infect Dis 2018; 5. doi: 10.1093/ofid/ofy146.
5. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing. 28th ed. CLSI supplement M100. Wayne, PA: Clinical and Laboratory Standards Institute; 2018.
6. Humphries RM, Hindler JA. Emerging resistance, new antimicrobial agents … but no test! the challenge of antimicrobial susceptibility testing in the current US regulatory landscape. Clin Infect Dis 2016; 63:83–8.
7. Labreche MJ, Graber CJ, Nguyen HM. Recent updates on the role of pharmacokinetics-pharmacodynamics in antimicrobial susceptibility testing as applied to clinical practice. Clin Infect Dis 2015; 61:1446–52.
8. Ellington MJ, Ekelund O, Aarestrup FM, et al. The role of whole genome sequencing in antimicrobial susceptibility testing of bacteria: report from the EUCAST Subcommittee. Clin Microbiol Infect 2017; 23:2–22.
9. Jones CL, Clancy M, Hornold C, et al. Fatal outbreak of an emerging clone of extensively drug-resistant Acinetobacter baumannii with enhanced virulence. Clin Infect Dis 2015; 61:145–54.
10. Hauser AR, Mecsas J, Moir DT. Beyond antibiotics: new therapeutic approaches for bacterial infections. Clin Infect Dis 2016; 63:89–95.