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BRIEF CASE

The Brief Case: Cough in an Immunocompromised Patient

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CASE

We report a case of a 78-year-old female with a medical history of metastatic breast cancer receiving chemotherapy who presented to the emergency department (ED) in the summertime complaining of dizziness and a cough. She had recently been seen by her primary care provider for shortness of breath, at which time a left-sided infiltrate was noted on chest X ray. A 5-day course of oral azithromycin had been prescribed for community-acquired pneumonia (CAP). She failed to improve and did not complete the treatment, because she presented to the ED prior to the end of therapy. She reported decreased appetite, fatigue, and shortness of breath. On physical examination, she was afebrile and tachycardic, and diminished breath sounds were auscultated in the left base. The complete blood count was notable for the absence of leukocytosis. Blood cultures were sent, and a chest X ray revealed hazy opacities in the left upper lobe (Fig. 1). No other testing, including respiratory or molecular tests, was conducted at that time, and the patient left the ED against medical advice.

Twenty-four hours later, the anaerobic set of blood cultures turned positive, and a Gram stain from the positive bottle revealed a tiny Gram-negative coccobacillus. Subsequent handling of the specimen was performed in a biosafety cabinet according to institutional protocol. An aliquot was set aside in case of need for rapid blood culture identification, but no rapid molecular tests were done due to the Gram stain morphology. Culture growth was present only on a chocolate agar plate, with no growth on sheep blood or MacConkey agar. Catalase and oxidase tests were weakly positive, which was concerning for possible *Brucella* or *Haemophilus* species. An agar slant urease test was positive in $<$20 min, and a satellite test did not show growth of an organism around *Staphylococcus aureus*, furthering the suspicion for *Brucella* infection, particularly since the patient's symptoms had failed to improve on therapy expected to treat *Haemophilus*. A chocolate agar slant was sent to the Michigan Department of Health and Human Services (MDHHS) for identification. When the blood culture turned positive, the patient was called, reported worsening respiratory symptoms, and was advised to present to the nearest ED. She denied any history of recent travel, ingestion of unpasteurized cheese/dairy, and exposure to farm animals. MDHHS later identified the bacterium as nontypeable *Haemophilus influenzae*. A repeat satellite test was found to be positive. The patient improved with inpatient antimicrobial treatment for bacteremic pneumonia and was discharged home.

DISCUSSION

This case highlights the fact that distinguishing between *Haemophilus* and *Brucella* is important not only clinically but also for maintaining the safety of laboratory personnel working with highly infectious organisms.

Haemophilus, a genus of Gram-negative coccobacilli important in both human and zoonotic disease, is transmitted via respiratory droplets or direct contact. The species appears as short (1.5-μm) coccobacilli, sometimes in pairs or chains, on a Gram stain (1).

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For answers to the self-assessment questions and take-home points, see https://doi.org/10.1128/JCM.00370-20 in this issue.

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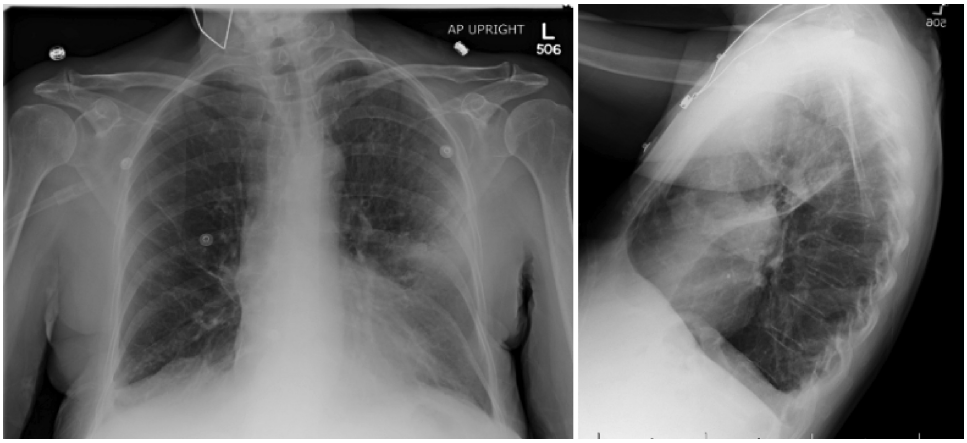

FIG 1 Anteroposterior and lateral films of the patient's chest radiography.

H. influenzae is particularly important as a cause of exacerbation of chronic obstructive pulmonary disease and CAP. Strains of *H. influenzae* may produce one of six distinct capsular polysaccharides (which provide the basis for serotype designation) or may be nonencapsulated. Nonencapsulated *H. influenzae* strains are referred to as nontypeable. An enriched medium, with factors X (hemin) and V (NAD/NAD^+) added, is required for the isolation of *H. influenzae*. The bacterium typically does not grow on sheep blood agar except around colonies of *Staphylococcus aureus* (known as a "satellite phenomenon"). This phenomenon occurs because both X and V factors are present in erythrocytes, and hemolysis by *S. aureus* releases both, thus allowing for growth on the blood agar plate (1). *H. influenzae* can also be identified through automated systems, including matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) systems.

In comparison, *Brucella*, also a small Gram-negative coccobacillus, is easily transmitted by aerosol and percutaneous inoculation, requiring a low infective dose (2). Most automated blood culture instruments can detect *Brucella* in $<$5 days, although detection is often delayed, typically 3 to 4 days, in contrast to $<$36 h for most other pathogens (2). While rapid methods to identify suspected agents of bioterror (such as MALDI-TOF) have been described, not all laboratories have this capability, and not all have validated the instrument for highly infectious agents (3).

Thus, adherence to the steps outlined in the American Society for Microbiology Sentinel Level Clinical Laboratory Guidelines for identification of a suspect agent of bioterrorism is particularly important in order to distinguish between the two species (2). First, small (0.4- by 0.8-μm), poorly staining Gram-negative coccobacilli are seen on a Gram stain. Following the identification of suspicious colonies, plates should be taped shut, and all further work should be performed in a biosafety cabinet. *Brucella* will typically grow on blood but, like *Haemophilus*, grows poorly and after prolonged incubation. Both grow equally well on chocolate agar; however, *Brucella* appears as pearl-white, nonhemolytic colonies, while *Haemophilus* appears as "morning dew drops." Neither species grows well on MacConkey agar, and both are oxidase and catalase positive. Finally, unlike most *Haemophilus* species, *Brucella* is rapidly urease positive, often within 15 min to 24 h. At that point, the presumed identification is *Brucella*, and further identification with automated systems should be avoided due to the high concentration of organisms as well as a high risk for aerosolization (2, 3).

Key components in the initial misidentification of this bacterium led the microbiology team to suspect *Brucella* rather than *Haemophilus*. First, the satellite test was initially negative, prompting the exclusion of *Haemophilus* as a potential organism. However, the satellite test has a number of problems that can result in false-negative results. Dealler et al. (4) estimated that approximately 10% of satellite tests are interpreted falsely or have to be repeated. One explanation is that *Haemophilus*

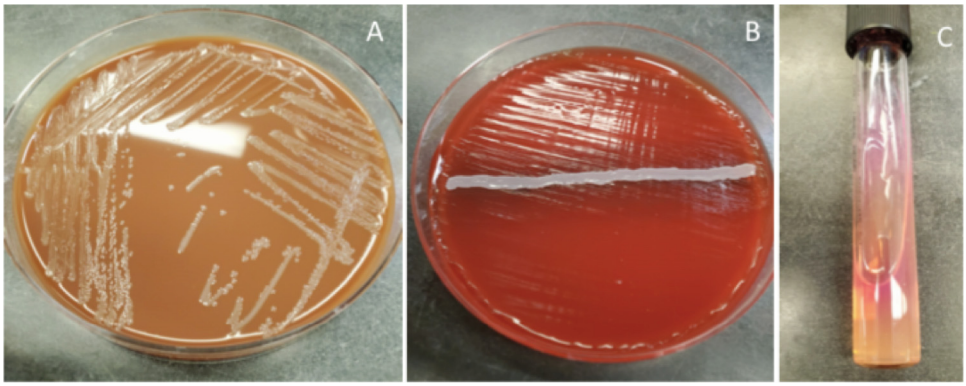

FIG 2 (A) Growth of colonies of *H. influenzae* on chocolate agar after 24 h. (B) Satellite growth of *Haemophilus* around a streak of *Staphylococcus aureus* on sheep blood agar after 24 h. (C) Positive urease slant test for *Haemophilus influenzae*.

generally has poor growth on the blood agar medium, despite the addition of *S. aureus*. In addition to this, the plate itself can be inadvertently inoculated with growth factors or contaminated with other bacteria. Finally, the test is not frequently performed and thus may be misinterpreted by clinical microbiology labs. Another component in the initial misidentification of the bacterium was the rapidly positive urease test. The urease test is used to determine if an organism can split urea using the enzyme urease. The test utilizes Christensen's urea agar, a medium that contains urea (2). If urease is present, ammonia is released, forming ammonium hydroxide, which alkalinizes the medium area of inoculum and changes the color from yellow-orange to pink (Fig. 2). Very few organisms can degrade urea in $<$4 h (considered to be a rapidly positive test). These organisms include *Proteus* spp., *Helicobacter* spp., and, importantly, *Brucella* spp. However, urease test positivity is variable in *Haemophilus* species, with a positive reaction occurring more classically with species such as *Haemophilus aegyptius* and *Haemophilus haemolyticus*. It is particularly important, however, that urease positivity in *H. influenzae* is in fact quite variable, and if a positive reaction should occur, it is typically less rapid. It is not known what percentage of *H. influenzae* strains have a positive urease reaction. Our case demonstrated a rapidly positive urease test, within 15 min, which would lead one to consider *Brucella* spp. rather than *Haemophilus* spp.

Finally, it is important to discuss the antimicrobial therapy that this patient received. The patient was initially treated with azithromycin and later completed inpatient therapy at an outside institution; thus, the duration and type of antibiotics she received there remain unknown. Susceptibility testing on the isolated *H. influenzae* strain revealed that it was negative for β-lactamase, and no further susceptibility testing was done on the isolate. Interestingly, the initial treatment course with azithromycin is recommended as standard-of-care therapy for patients without comorbidities or risk factors for resistant pathogens (5). However, given the patient's active malignancy and immunosuppressive therapy (and the presence of bacteremia), single-agent oral therapy would not be appropriate, and combination therapy, including a β-lactam (such as a third-generation cephalosporin) would be advised. With regard to macrolide resistance, *H. influenzae* isolates worldwide show low-level intrinsic resistance that acts in combination with mutational and acquired resistance. Resistance typically occurs in the presence of an intrinsic or acquired efflux pump, or through mutations in 23S rRNA and other ribosomal proteins (6). The clinical significance of macrolide resistance to *H. influenzae* is debated, since MICs are generally relatively low for azithromycin (from 0.1 to 4 μg/ml), and antibiotic failure is more common when the isolate demonstrates higher MICs (6). However, antimicrobial susceptibility testing for *Haemophilus* is not typically done, since the *in vitro* testing demonstrates considerable inter- and intralaboratory variation and does not typically reflect the patient's outcome (6).

We report a case of *Haemophilus* infection that was rapidly urease positive, with an

initially negative satellite test—both characteristics that would typically be concerning for *Brucella* identification. Furthermore, the patient failed treatment with an antimicrobial generally expected to treat *Haemophilus*. Nevertheless, appropriate handling of the specimens via institutional protocols was of the utmost importance. Clinical microbiologists must have an understanding of the technical principles that are necessary for the recognition and differentiation of a potential biological threat from other species.

SELF-ASSESSMENT QUESTIONS

1. An organism grows well on chocolate agar but poorly or not at all on blood agar. What is the first mandatory step when a Gram stain reveals a tiny, Gram-negative coccobacillus?
   a. Tape the plate, and perform further workup within a biosafety cabinet.
   b. Identification using automated systems
   c. Catalase test
   d. Oxidase test
2. What is the most common mode of transmission of *Brucella* among laboratory workers?
   a. Contact
   b. Ingestion
   c. Aerosol
   d. Fomites
3. What is the result of the urease test in strains of *H. influenzae*?
   a. Rapidly positive in $<$4 h
   b. Positive at 24 h
   c. Negative
   d. Variable

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