A Case of *Bordetella holmseii* Endocarditis in an Asplenic Pediatric Patient

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INTRODUCTION

A 9-year-old male patient with congenital dextrocardia and congenital asplenia history was seen in the emergency room due to parental concern for dehydration as a result of gastritis of five day duration. Previously, in 2006, the patient had mitral valve replacement and a pace maker implanted. The patient was admitted as a result of the current emergency room visit when the physician suspected endocarditis due to the patient's asplenia, artificial heart valve, and fever. An echocardiogram was performed. The echocardiogram was negative and showed no vegetations. A CBC was ordered on the patient. Results showed an elevated WBC count of 19.4 x 10^3/µL. (Table 1) Ten blood cultures were drawn during the patient's admission; three were positive. The first positive blood culture grew bacteria after three days incubation in the aerobic bottle. The second positive blood culture was drawn six days later and took 3.6 days for growth to occur. The third positive blood culture, drawn 4 days after the second, took 2.5 days to become positive. Blood culture Gram stain results showed presence of small to moderate sized gram negative rods. Subsequent plate growth indicated that the organism grew best on Chocolate agar, appearing after 72 hours and was catalase negative. No growth was observed on MacConkey agar at three days. bioMérieux automated VITEK® 2 identification of the organism, grown from the blood, suggested *Bordetella* species. Organism identification was also obtained using MALDI-TOF mass spectrometry (VITEK® MS) instrumentation, which was undergoing validation studies at the time, and was identified as *Bordetella holmseii*. *Bordetella holmseii* identification was confirmed on this pediatric patient's specimen at Michigan Department of Community Health state diagnostic laboratory using 16S rRNA gene sequencing and analysis.

Table 1. Patient's Admission CBC Results. WBC differential was manual.

CBC Parameters/units		Results/Flag
WBC	10^3/µL	19.42
SEG	%	74/H
BAND	%	6
LYM	%	7 L
MONO	%	10
EOS	%	2
BASO	%	0
META	%	1
RBC	10^6/µL	4.61
HGB	g/dL	12.5
HCT	%	36.2
MCV	fL	78.5/L
MCH	pg	27.1
MCHC	g/dL	34.5
RDW	%	13.9
Platelets	10^3/µL	332
MPV	fL	11.5/H

Comments: Few Schistocytes, Few Spherocytes and Few Echinocytes

MICROBIOLOGY

Bordetella holmseii is characterized as a predominantly small sized Gram negative coccoid to short bacillus (Figure 1), that is biochemically asaccharyolytic, non-motile, and catalase and oxidase negative.[11] *Bordetella holmseii* can also be recognized by production of a light brown diffusible pigment (may appear as alpha hemolysis on sheep blood agar), especially when grown on solid media such as heart infusion tyrosine agar at

35°C.[1] *Bordetella holmseii* is easily differentiated from *Bordetella pertussis,* based on characteristics such as its ability to grow on routine media (Blood and Chocolate agar) (Figure 2), and further from *Bordetella bronchiseptica* and *Bordetella avium* because it is oxidase and motility negative. Differentiation from *Bordetella parapertussis* is evident based on the urease result; *Bordetella holmseii* is negative and *Bordetella parapertussis* positive. *Bordetella holmseii* may also closely resemble and be confused with *Acinetobacter* species. *Bordetella holmseii's* small coccobacillus to rod shaped appearance on Gram stain, no growth on MacConkey agar at three days, catalase negative, and pigment production results help in differentiation from *Acinetobacter* species. *Acinetobacter* is primarily a Gram negative coccobacillus without rod forms, usually grows on MacConkey agar at 48 hours, is catalase positive, and is negative for brown pigment production.

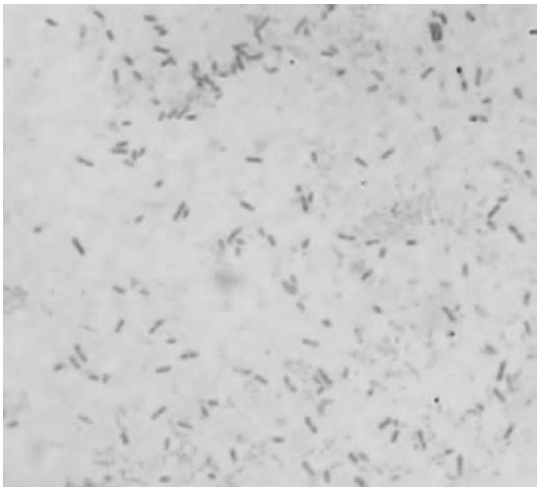

Figure 1. Bordetella holmseii Gram stain from Chocolate agar (1000X)

CLINICAL FEATURES, EPIDEMIOLOGY, AND TRANSMISSION

Bordetella holmseii was first recognized in 1983 and identified and put in the genus *Bordetella* in 1995 (previously CDC non-oxidizer NO-2).[11] The natural habitat of *Bordetella holmseii* remains unknown despite suspicion that it may be a colonizer in the human respiratory tract.[10,12] Mode of acquisition is also unknown; person to person transmission has been suggested but not proven.[4,6] It is an infrequent and opportunistic isolate primarily occurring in compromised patients causing non-respiratory infections, including septicemia and endocarditis, and has also been associated with pertussis-like respiratory infections.[12] Asplenia appears to be a risk factor for serious infection with this organism, with one study reporting that 85% of patients diagnosed with *Bordetella holmseii* bacteremia had either anatomical or functional asplenia.[7] Other conditions that may predispose a patient or serve as a risk factor for infection with *Bordetella holmseii* include sickle cell anemia, hemolytic anemia, heart valve replacement or repair, lymphoma, and Hodgkin's disease.[7,11]

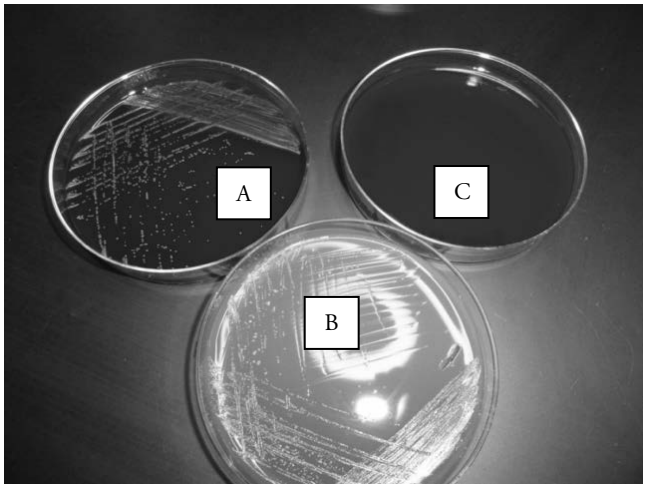

Figure 2. Growth of Bordetella holmseii at 72 hours. Note pinpoint growth on blood agar (A) and Chocolate agar (B) and no growth on MacConkey agar (C).

TREATMENT

Standardized treatment data is not readily available due to the rare isolation of *Bordetella holmseii* as a cause of infection. In the case presented here, patient susceptibility studies were performed using E-test method and showed the organism was resistant to third generation cephalosporins (cefixime) at ≥ 32 ug/ml. Additional *Bordetella* susceptibility testing results are listed in Table 2. In this case, susceptibility studies are reported using only MIC values and not the interpretive breakpoints of susceptible (S), intermediate (I), and resistant (R) because non-standardized (not Clinical and Laboratory Standards Institute) methods were employed. All susceptibility testing results are considered non-standardized when the procedure has not been performed and verified previously for a given antimicrobial-organism combination using a specific method. The report should have a qualifying text explanation for interpretation indicating that "no standardized susceptibility breakpoints are available". In this patient, ceftriaxone would be considered resistant

regardless.

Azithromycin was prescribed for this pediatric patient and given as an oral agent along with four weeks of intravenous meropenem. The patient remains on azithromycin therapy indefinitely and appears to be responding favorably. Previous publications have reported high MICs for β-lactam agents with *Bordetella holmseii*, including third generation cephalosporins, suggesting that these agents are not ideal for treatment.[9] However, susceptibility against ceftazidime in an aortic valve infection from a 2012 case study in an adult, had contradictory results with findings indicating a low MIC value for ceftazidime.[3] Therefore, due to unpredictable sensitivity results, in cases where *Bordetella holmseii* is identified, susceptibility studies should be performed.

Table 2. E-Test Susceptibility Results for Bordetella holmseii.

Antimicrobial Agent	MIC (ug/ml)
Ampicillin	3
Ceftriaxone	≥32
Ertopenem	0.125
Gentamicin	0.75
Levofloxacin	0.125
Meropenem	0.064
SXT	0.094
Azithromycin	0.50
Ceftazidime	1.5

DISCUSSION

Physical exam and a patient's symptoms may lead a clinician to suspect endocarditis. However, confirmation of endocarditis is based on the presence of bacteremia, with multiple blood cultures required to assist with this diagnosis. Often, questions as to how many blood culture sets are needed and what timing is suggested for them to be drawn to confirm endocarditis diagnosis, arise. In general, for suspected acute endocarditis, three sets of blood cultures are collected from three separate venipuncture sites in a 1-2 hour time period, prior to starting antibiotic therapy. For subacute endocarditis, three sets of blood cultures are collected from separate venipuncture sites in a 24 hour period. If all remain negative at 24 hours after collection, then the physician should order three additional sets.[2,8]

Another question that clinicians and laboratorians alike may ask is, why is *Bordetella holmseii* not isolated more frequently from respiratory specimens. Low pathogenicity, lack of organism awareness, slow organism growth, along with cephalexin presence in routine transport medium used for the isolation of *Bordetella pertussis* or other respiratory pathogens may account for the small numbers of *Bordetella holmseii* infections found and explain why it is not isolated more frequently in respiratory infections. Further, Mazengia, et.al. reported that recovery of *Bordetella holmseii* was inhibited when grown on Bordet Gengou selective medium.[5] In patients with respiratory symptoms resembling whooping cough, routine specimen collection procedures, using antibiotic-containing transport medium for *Bordetella pertussis* recovery, would most likely not support growth and isolation of *Bordetella holmseii*.

An unusual pathogen report, such as *Bordetella holmseii* sepsis in a compromised patient, leaves clinicians wondering what to expect regarding patient prognosis. *Bordetella holmseii* has been recognized as a rare opportunistic pathogen, primarily causing infection in immunocompromised patients, with only nineteen cases of infection reported in the literature from 1995 through 2000.[5] Bacteria that are encapsulated have a reputation and predilection for virulence and are seen especially in cases of sepsis; however, evidence of the presence of a capsule in *B. holmseii* has yet to be determined and its typical lack of fulminant progression also supports this theory.[6,7] *Bordetella holmseii* respiratory infections most often resolve spontaneously; other more serious infections, including bacteremia, septic arthritis, and endocarditis, generally have favorable outcomes with proper therapy.[6,7]

A final consideration and important point of discussion for any laboratory when isolation of an unusual pathogen is suspected is a reminder that routine procedures and methods for identification should be scrutinized in these cases. A 2010 study on four asplenic children with *Bordetella holmesii* bacteremia infection showed incorrect identification by bioMérieux Vitek with 99% probability as *Acinetobacter lwoffii*.[7] Another aortic valve *Bordetella holmseii* infection, from a 2012 case report, showed API 20 NE strip presumptive identification as *Acinetobacter* species with 80% probability.[3] This isolate was confirmed as *Bordetella holmseii* by molecular identification.[3] Other routine identification procedures have given erroneous

identifications for infections with *Bordetella holmseii* in blood and joint fluid specimens and include misidentifications such as *Moraxella lacunata* and *Aeromonas salmonicida/Oligella urealytica.*[6] Laboratorians should be cautioned that the database of automated systems may not include rare and unusual organisms such as *Bordetella holmseii*. Catalase testing and pigment production are important biochemical results that also may be omitted from instrument systematic identification schema. In addition, because automated systems tie antibiotic susceptibility testing results to correct organism identification, antibiotic susceptibility determination must be performed using alternative methods.

CASE CONCLUSIONS

Clinicians should be aware of the increased chances of bacteremia with *Bordetella holmseii* in febrile, asplenic patients. Microbiologists should be alerted to the possible presence of this infrequently isolated organism when slow-growing GNRs or GNCB are seen in this same patient population. *Acinetobacter* identification with unusual biochemical or growth characteristics should be questioned. An awareness of these clinical criteria, identifying biochemical results, and the possibility of misidentification via automated or other systems will prepare vigilant clinical laboratorians to properly recognize, identify, and thereby correctly diagnose this unusual pathogen. The use of 16S rRNA gene sequence analysis was reliable for confirmation in this case. Gene sequence analysis testing is offered by many state health department and other reference laboratories and may be helpful in identification of uncommon or difficult to identify isolates. In the future, implementation of mass spectrometry, as it becomes available in more clinical laboratories, will also have a significant impact on identification of unusual and rare organisms as cause of infection.

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