Bordetella holmesii Bacteremia in Asplenic Children: Report of Four Cases Initially Misidentified as Acinetobacter lwoffii

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Bordetella holmesii is a fastidious Gram-negative rod that was initially identified in 1995. It causes bacteremia, predominantly among patients with anatomical or functional asplenia. We report four cases of B. holmesii bacteremia in asplenic children occurring within the last 4 years. In all cases, B. holmesii was misidentified by an automated system as Acinetobacter lwoffii.

Four male adolescent patients, aged between 13 and 17 years, presented with mild febrile illness but no other clinically significant complaints within a period of 3.5 years. Three were suffering from sickle cell disease; two of them had undergone splenectomy. In the fourth patient, elective splenectomy was performed for an autoimmune hemolytic anemia. No epidemiological link was found between the four patients.

At the emergency department, they appeared well and had no remarkable findings on physical examination or initial laboratory workup. Blood cultures all grew Gram-negative bacilli. Two of the patients received intravenous (i.v.) ceftriaxone for 2 and 7 days, respectively. The remaining patients were treated with i.v. ceftriaxone followed by oral ciprofloxacin for a total of 10 days.

The clinical outcome was uniformly favorable. Clinical and laboratory findings are summarized in Table 1.

The Gram-negative bacilli isolated from the blood cultures (Bactec 9240, Peds Plus, and Plus Aerobic bottles; BD Diagnostics Inc., Sparks, MD) were small and coccoidal. They tested negative for catalase, oxidase, nitrate reduction, and urease activity as well as indole and oxidative-fermentative (OF) glucose media. The mean incubation time was 40 h (range, 30 h to 47.3 h). Colonies grew well at 24 h when inoculated on 5% sheep blood agar plates and incubated in 5% CO₂, whereas only three strains showed very limited growth after 48 h on MacConkey agar. While they were growing, they all produced a diffusible brown pigment which could have been mistaken for a hemolysis on a sheep blood agar plate. We were unable to identify the isolates through routine laboratory protocols and inoculation into API 20E and API 20NE strips (bioMérieux Inc.). We finally used the Gram-negative card on a Vitek2 automated system (bioMérieux Inc.), which reported Acinetobacter lwoffii with 99% probability (excellent identification confidence level) for all isolates. Some basic characteristics did not match this result. A. lwoffii usually grows well on MacConkey agar, has a positive catalase activity, and does not produce any pigment. All isolates were subsequently identified as Bordetella holmesii by 16S rRNA gene sequence analysis performed at Quebec’s Laboratory of Public Health, as previously described (2).

MICs determined by Etest on Mueller-Hinton agar were ≤0.25 μg/ml for piperacillin, ticarcillin-clavulanate, ceftazidine, meropenem, ciprofloxacin, trimethoprim-sulfamethoxazole, and polymyxin E; MICs for gentamicin and tobramycin were 2 μg/ml for one isolate and ≤0.25 μg/ml for the remaining three. Although all patients were treated with ceftriaxone, susceptibility testing for ceftriaxone was not performed.

The genus Bordetella belongs to the family of Alcaligenaceae and currently comprises eight species, including B. holmesii. Bordetella pertussis is the causative agent of whooping cough, while Bordetella parapertussis and Bordetella bronchiseptica are also implicated in respiratory tract infections in humans. Bordetella avium is considered a strict avian pathogen but has recently been isolated in the sputum of cystic fibrosis patients and others with respiratory disease (9, 19). Bordetella hinzii was found to colonize the respiratory tract in poultry and also in humans with cystic fibrosis. It can occasionally cause infection in immunocompromised patients (5, 19, 23). Bordetella trematum has been isolated from ear and wound infections and from a diabetic patient with a leg ulcer (3, 22). Bordetella petrii has been documented in patients with cystic fibrosis (19), in a patient with mandibular osteomyelitis, and in another with mastoiditis (7, 20). It is noteworthy that, in this last patient, the Vitek2 automated system misidentified the organism as Pseudomonas fluorescens. A recently proposed species is Bordetella ansorpii, which was first isolated in pus from an epidermal cyst and was also found to cause bacteremia in an immunocompromised patient (6, 10).

B. holmesii was first described in 1995 as a cause of septicaemia in 15 patients, including at least three children with asplenia, without any further clinical information (24). The first detailed clinical case report was published later that year with the description of a 12-year-old male who had a history of...
spleenectomy for an idiopathic thrombocytopenic purpura. He presented with fever but had an otherwise-normal physical examination. He quickly responded to i.v. ceftriaxone (11). Since then, \textit{B. holmesii} has been reported as a cause of bacteremia, endocarditis, and pneumonia, mainly in immunocompromised and, more particularly, in asplenic patients (4, 8, 12, 13, 15, 18, 21). In the largest published series so far, describing 26 patients with \textit{B. holmesii} bacteremia, 85% had anatomical or functional asplenia (18). The clinical course usually remained uneventful, and patients tended to recover without complications. However, an immunocompetent adolescent with \textit{B. holmesii} bacteremia and lobar pneumonia developed empyema. He eventually evolved toward pulmonary fibrosis (17). In most published articles, failure of identification by commercial systems and identification ultimately made possible by using 16S rRNA gene sequence analysis are reported. None of the other studies clearly mentioned systematic misidentification as \textit{A. lwoffii} or another bacterium using an automated identification system.

\textit{B. holmesii} has been isolated from cultures of nasopharyngeal specimens in 0.6% of U.S. patients with pertussis-like symptoms (25) but was not demonstrated using \textit{B. holmesii}-specific PCR on nasopharyngeal swabs from Dutch and Finnish patients with the same symptoms (1). The role of \textit{B. holmesii} in respiratory tract colonization or infection remains to be elucidated. There is no known reservoir for \textit{B. holmesii}. In respiratory tract colonization or infection remains to be elucidated. There is no known reservoir for \textit{B. holmesii}.

The above-described clinical cases and results of microbiological analysis support the growing evidence for the role of \textit{B. holmesii} as a pathogen among asplenic patients and suggest that it should always be considered, especially when the Vitek2 automated system reports \textit{A. lwoffii}.

\begin{table}[h]
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\begin{tabular}{|l|c|c|c|}
\hline
Parameter & Result for patient: & & \\
\hline
Age (yr) & Sickle cell anemia & Sickle cell anemia & Sickle cell anemia \\
Medical condition & Autoimmune hemolytic anemia & & \\
\hline
Spleenectomy & Yes & No & Yes \\
\hline
Age at splenectomy (yr) & 3 & 6 & 3 \\
Temp (°C, maximum) & 39.7 & 39.5 & 39.5 \\
Duration of fever (days) & 3 & 1 & 3 \\
Treatment & Ceftriaxone for 2 days & Ceftriaxone for 7 days & Ceftriaxone for 7 days \\
& followed by ciprofloxacin for 3 days & followed by ciprofloxacin for 5 days & followed by ciprofloxacin for 5 days \\
\hline
Blood hemoglobin level (g/dl) & 9.7 & 12.1 & 9.5 & 11.7 \\
Platelet count/mm\(^3\) & 321,000 & 677,000 & 261,000 & 396,000 \\
White blood cell count/mm\(^3\) & 5,590 & 17,280 & 15,640 & 11,560 \\
Neutrophils (%) & 46 & 83 & 81 & 87 \\
\hline
Chest X ray & Normal & Normal & Tc/Ga scintigraphy, normal & Tc/Ga scintigraphy, normal \\
Other test results & Cardiac U/S, normal & 4 days & 7 days & 7 days \\
Length of hospital stay & Not admitted & 7 days & & \\
\hline
\end{tabular}
\caption{Clinical characteristics and laboratory findings for the 4 patients}
\end{table}


