SHORT COMMUNICATION

Bordetella holmesii: initial genomic analysis of an emerging opportunist

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An inexpensive draft genome of Bordetella holmesii reveals unsuspected phylogeny, virulence factors of this emerging pathogen, and a predicted toluene-4-monoxygenase.

Keywords
Bordetella holmesii; genome; asplenia; opportunistic.

Abstract
Bordetella holmesii is an emerging opportunistic pathogen that causes respiratory disease in healthy individuals and invasive infections among patients lacking splenic function. We used 16S rRNA gene analysis to confirm B. holmesii as the cause of bacteremia in a child with sickle cell disease. Semiconductor-based draft genome sequencing provided insight into B. holmesii phylogeny and potential virulence mechanisms and also identified a toluene-4-monoxygenase locus unique among bordetellae.

Bordetella holmesii is a brown pigment-producing Gram-negative coccobacillus that is increasingly recognized as a cause of invasive disease in immunocompromised patients (Weyant et al., 1995; Shepard et al., 2004). Reports have linked B. holmesii to bacteremia, meningitis, pneumonia, pericarditis, endocarditis, and other clinical syndromes including pertussis-like illnesses (Mazengia et al., 2000; Shepard et al., 2004; Mooi et al., 2012). Although most cases are sporadic, a cluster of B. holmesii bacteremia among children was reported in 2010 (Layton & Weiss, 2010). Definitive identification of B. holmesii can be difficult using routine phenotypic methods, and sequence-based approaches are frequently required (Russell et al., 2001). As only limited genetic information is available to help understand the unusual predisposition of B. holmesii to cause invasive disease in asplenic hosts including those with sickle cell disease (McCavit et al., 2008; Gross et al., 2010), we constructed a draft whole-genome sequence of a B. holmesii clinical isolate and used this information to analyze putative virulence determinants.

A 7-year-old girl with sickle cell (hemoglobin SS) disease presented with fever and tachycardia. She had been receiving routine oral penicillin prophylaxis. Physical examination did not reveal a focal infection. Laboratory studies revealed a peripheral leukocytosis with a neutrophil predominance and an elevated platelet count. Blood samples were taken for culture, and empiric therapy was initiated with intravenous ceftriaxone. Fevers continued during the first day of hospitalization and then resolved. Two blood cultures were positive for a slow-growing (> 48 h), Gram-negative...
microorganism on chocolate and sheep blood agar with poor growth on MacConkey agar. The isolate was oxidase-negative and produced a diffusible brown pigment. Automated identification systems failed to conclusively identify the pathogen due to its asaccharolytic nature. Based upon these data, *B. holmesii* was suspected. Antimicrobial susceptibility testing by *E*-test revealed minimum-inhibitory concentrations (in µg mL⁻¹) as follows: penicillin, > 32; cefoxitin, > 256; ceftazidime, 1; levofloxacin, 0.032; erythromycin, 0.125; azithromycin, 0.047; trimethoprim-sulfamethoxazole, 0.125. Using 16S rRNA gene sequencing as described (Schuetz et al., 2012), we confirmed the isolate as *B. holmesii* (1525-bp, 100% match to *B. holmesii* type strain ATCC51541ᵀ). Subsequent blood cultures were negative, as was a cerebrospinal fluid culture. Echocardiography revealed neither vegetations nor valvular abnormalities. The patient improved and was discharged on oral levofloxacin.

To better understand the biology of this unusual organism, we undertook a sequencing and assembly project to generate a draft genome of the strain from this case (*B. holmesii* 44057). Bacterial genomic DNA was purified using a commercial kit (DNeasy; Qiagen) and quantified by spectrophotometry. Library preparation and sequencing using the Ion Torrent Personal Genome Machine 316 chip were performed by the Columbia Genome Center using manufacturer-specified protocols. A single run generated 2.8 × 10⁶ reads (mean length, 118 bp; 3.3 × 10⁸ total bases). Sequence assembly was performed with CLC Genomics Workbench (version 5.0.1), generating 299 total contigs (> 250 bp each). The total size of the contigs was 3.43 Mbp, which may be less than the actual genome size, as the assembler was unable to match all bases into contigs > 250 bp (95% of reads, 94% of bases assembled). The GC % for all contigs was 62.9%, consistent with the value of 61.9% reported for the *B. holmesii* type strain (Weyant et al., 1995).

After removal of a single contig with > 5% ambiguous bases, the draft *B. holmesii* genome was annotated (3742 predicted coding sequences, 44 RNAs; see Supporting Information associated with this manuscript) and compared to known *Bordetella* sequences using the Rapid Annotation using Subsystem Technology (RAST) server (Aziz et al., 2008). We focused our initial analysis on known *Bordetella* virulence factors and gene clusters that might be unique to *B. holmesii* with the intention of performing more detailed genomic analysis as more complete and widely representative sequence data become available.

Early phylogenies based on the 16S rRNA gene indicated that *B. holmesii* is most closely related to *Bordetella pertussis* (Gerlach et al., 2001). However, subsequent analysis based on multiple housekeeping genes demonstrated that *B. holmesii* is a closer relative of *Bordetella avium* and that it had likely acquired both its 16S rRNA gene and iron-uptake island by lateral transfer from *B. pertussis* (Diavatopoulos et al., 2006). To help resolve these discrepancies, we employed the program INSID, which utilizes raw, unprocessed sequencing reads to create a whole-genome phenogram. The resulting tree is congruent with taxonomies that place *B. holmesii* close to *B. avium* (Fig. 1). To survey the similarity of *B. holmesii* to other species on a gene-by-gene basis, we used a BLAST-based approach to assign a best hit for each open reading frame (ORF) in the draft genome. At the nucleotide level, 42% (1576/3742) of genes had a best hit in *B. avium* while only 11% (408/3742) had a best hit in *B. pertussis*. Amino acid comparisons showed even stronger similarity with 61% *B. avium* best hits and 4% *B. pertussis* best hits. These data are consistent with a closer overall relationship between *B. avium* and *B. holmesii*.

Many potential *Bordetella* virulence factors have been previously described, some of which are present in the nonclassical bordetellae (i.e. not *B. pertussis*, *B. parapertussis*, or *B. bronchiseptica*; Gross et al., 2010). Consistent with prior findings (Gerlach et al., 2004; Horvat & Gross, 2009), the draft genome of *B. holmesii* 44057 encodes ORFs with significant similarity to the *bvg* virulence regulatory system (RAST 35814.9.peg.2521-2524). Likewise, the alcaligin siderophore biosynthesis operon, part of the laterally transferred iron-uptake island (Diavatopoulos et al., 2006), was present in this strain (35814.9.peg.84-96). Bordetella holmesii filamentous hemagglutinin, encoded by *thaB*, has been described previously (Link et al., 2007). Following manual curation, the *B. holmesii* 44057 *thaB* (35814.9.peg.2499) predicted amino acid sequence was found to be 99% identical to the published sequence. Numerous components of flagellar biosynthesis, types II and III secretion systems, and capsular polysaccharide synthesis and export were identified, although the existence of an

![Fig. 1 Phenogram of representative whole genomes from the genus Bordetella. Whole-genome similarity between strains was calculated using the program INSID. Publically available whole genomes were shredded into 200 000 segments consisting of 400 base pairs each and compared to the unprocessed sequencing reads generated by the Ion Torrent platform for Bordetella holmesii. The pairwise similarity matrix was based on comparisons using a 15-megabase (Mb) query and 5-Mb reference. For bootstrap values, each set of genomic fragments/reads was resampled 100 times with replacement, generating 100 new read sets, and 100 new similarity matrices. All trees were generated using the UPGMA algorithm in PAUP. Note that the phenogram topology is remarkably robust to bootstrap resampling. Branch length is proportional to average pairwise similarity as calculated by INSID.](https://academic.oup.com/femspd/article-abstract/67/2/132/2398864/133)
A novel toluene-4-monoxygenase locus in <i>Bordetella holmesii</i> 44057 compared to the homologous region from <i>Gordonia polyisoprenovorans</i> VH2. The area of significant nucleotide identity between the two loci is indicated by the bracketed region. There was no detectable nucleotide similarity in the flanking regions. Homologous genes are indicated using the same color, and percentages above gene arrows represent values for amino acid similarity between homologs. Note that there is low-level similarity at the amino acid level and in gene annotation in some flanking genes, suggesting insertion in a similar genomic location after the putative horizontal gene transfer event. <i>Pseudonocardia dioxanivorans</i> CB119 (not pictured) has at least three separate loci with strong nucleotide similarity to this locus. The largest of these regions has 65% nucleotide identity but does not span the entire locus.

**Fig. 2**

Bordetella holmesii genomics P.J. Planet et al.

This whole-genome shotgun project has been deposited at DDBJ/EMBL/GenBank under the accession ANGE.

Determination of complete genome sequences of several clinical isolates of <i>B. holmesii</i> is underway and will provide additional data regarding diversity within this species and its relationship with other members of the bordetellae (Preston et al., 2004).

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References


Supporting Information

Additional Supporting Information may be found in the online version of this article:

Data S1. RAST annotation of the Bordetella holmesii 44057 genome (GENBANK format).

00000000. The version described in this paper is the first version, ANGE01000000. Raw sequence reads are publicly available through the NCBI Sequence Read Archive (BioProject ID: 158371; accession number SRX201839), and the B. holmesii 44057 RAST annotation is included as Supporting Information with this manuscript.