

First report of *Bordetella ansorpii* and *Bordetella trematum* in clinical samples: emergence of uncommon species in Brazil

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Bacteria of the genus *Bordetella* are of great importance in human and veterinary medicine due to their ability to colonize the respiratory tract, causing a wide range of pulmonary and bronchial infections. In the 1990s, *Bordetella trematum* was first isolated from wounds and ear infections in humans in Europe and the United States, typically associated with tissue infections. In 2005, a new species, *Bordetella ansorpii*, was proposed after the isolation of a single strain from the purulent exudate of an epidermal cyst in a patient in Korea. Both species are considered uncommon and, until now, there were no reports describing their occurrence in Brazil. This study describes the first report of *B. ansorpii* and *B. trematum* isolated in 2025 at the Adolfo Lutz Institute (IAL), São Paulo, Brazil. Two clinical samples were analyzed: (i) secretion from a gluteal abscess from a 75-year-old male patient and (ii) a fragment of cutaneous tissue from a 42-year-old male patient, both from the state of São Paulo. Specimens were cultured on charcoal agar supplemented with 10% horse blood and cephalixin and incubated at 35-37°C. Phenotypic identification was performed by biochemical tests and by MALDI-TOF mass spectrometry (Bruker Daltonics), and genotypic identification by whole genome sequencing (WGS) using the PacBio Sequel IIe platform with SMRT Cell 8M. SNP analysis was performed using CSI Phylogeny 1.4, and the resulting phylogenetic tree was visualized in Microreact (<https://microreact.org/>). Comparative genomes of *Bordetella* species for SNP analysis were obtained from the NCBI database. Both isolates (i) and (ii) showed colonies suggestive of the genus *Bordetella* after 24 hours of incubation and also showed growth on common agar, blood agar, and MacConkey agar. Regarding biochemical tests, isolate (i) showed positive results for catalase, Simmons citrate, urease, and nitrate reductase and negative for oxidase, while isolate (ii) was positive for catalase, oxidase, and Simmons citrate and negative for urease and nitrate reductase. Regarding MALDI-TOF analysis, isolate (i) was identified only at the genus level (*Bordetella* sp.), with a score value lower than 2.0. In contrast, isolate (ii) was identified as *B. trematum*, with a score value >2.0, indicating high reliability in identification. Genomic analysis confirmed the identification of *B. trematum* and enabled the precise identification of the gluteal abscess isolate (i) as *B. ansorpii*. Whole genome sequencing (WGS) generated unique contig assemblies for both isolates. The genome size of *B. ansorpii* was 6,321,897 bp (168× coverage), while that of *B. trematum* was 4,433,578 bp (180× coverage). Comparative SNP analysis using genomes from different countries revealed substantial genetic divergence in both species

(8,600–44,000 SNPs for *B. trematum* and 1,118–60,059 SNPs for *B. ansorpii*), indicating moderate to high genomic variability. This study reports the first identification of *B. ansorpii* and *B. trematum* in Brazil, reinforcing the need to expand diagnostic protocols to include non-classical *Bordetella* species, highlighting the essential role of MALDI-TOF mass spectrometry and whole-genome sequencing in the accurate identification of species. Further studies integrating clinical, microbiological, and genomic data are needed to clarify the role of uncommon species in human infections.

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