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RESEARCH NOTE

Identification of *Bordetella* spp. in respiratory specimens from individuals with cystic fibrosis

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ABSTRACT

Bordetella spp. are not normally included when considering the opportunistic bacterial species that are typically involved in respiratory tract infections in individuals with cystic fibrosis (CF). By using a combination of bacterial genotyping and 16S rDNA sequencing, *Bordetella* spp. were identified in cultures obtained from 43 individuals with CF. Most (n = 23) patients were infected with *Bordetella bronchiseptica/parapertussis*; five were infected with *Bordetella hinzii*, four with *Bordetella petrii*, three with *Bordetella avium*, and eight with unidentified *Bordetella* spp. Consideration should be given to the presence of these organisms in the evaluation of CF sputum cultures.

Keywords 16S rDNA sequencing, *Bordetella* spp., cystic fibrosis, rep-PCR, respiratory tract infection, sputum

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Cystic fibrosis (CF) is an autosomal recessive disease, characterised by defective chloride ion channels that result in multi-organ dysfunction, most notably affecting the respiratory tract. The alteration in the pulmonary environment is associated with increased susceptibility to bacterial infection. Recent advances in bacterial taxonomy and improved microbial identification systems have led to an increasing recognition of the diversity of bacterial species involved in CF lung infection. Many such species are opportunistic human pathogens, some of which are rarely found in other human infections [1]. Processing of CF respiratory cultures therefore employs selective media and focuses on detection of uncommon human pathogens. In recent years, the use of molecular methods (e.g., species-specific PCRs and assays based on identifying restriction fragment length polymorphisms) has complemented the diagnostic capacity of routine phenotypic analyses. The present study describes the use of molecular methods to identify *Bordetella* spp. in respiratory tract cultures from 43 patients with CF.

The 43 bacterial isolates included in the study were recovered between June 2001 and June 2007 from sputum cultures obtained from 43 CF patients receiving care in 19 treatment centres in the USA. All isolates were sent to the *Burkholderia cepacia* Research Laboratory and Repository (BcRLR; University of Michigan, Ann Arbor, Michigan, USA) for microbiological evaluation. Reference strains comprised *Achromobacter xylosoxidans* ATCC 9220, *Alcaligenes faecalis* ATCC 337, *Alcaligenes faecalis* subsp. *faecalis* LMG 1229^T, *Alcaligenes* sp. ATCC 21030, *Bordetella avium* LMG 1852^T, *Bordetella bronchiseptica* LMG 1232^T, *Bordetella hinzii* LMG 13500, *Bordetella holmesii* LMG 15945^T, *Bordetella parapertussis* LMG 14449^T, *Bordetella pertussis* LMG 14455^T and *Bordetella trematum* LMG 13506^T. *B. pertussis* was grown on Regan–Lowe charcoal agar (BBL, Franklin Lakes, NJ, USA). All other bacterial isolates were grown aerobically on plates containing Mueller–Hinton broth (Becton Dickinson, Cockeysville, MD, USA)

supplemented with agar 1.6% w/v, and were incubated at 32°C for 24–48 h. DNA was prepared from bacterial cultures as described previously [2]. A PCR targeting the 16S rDNA of *A. xylosoxidans* was performed as described previously [3]. Bacterial genotyping was performed using rep-PCR with the BOX A1R primer (BOX-PCR) as described previously [4], with cluster analyses performed by the unweighted pair-group method with arithmetic averages (UPGMA). 16S rDNA PCR amplification, sequencing and editing were performed as described previously [2]. Isolates were identified to the genus level if their edited and assembled 16S rDNA sequence identity compared with genera identified in the GenBank database was >95%; isolates were tentatively identified to the species level if the sequence identity was ≥99%. All 16S rDNA sequences determined in this study were deposited in GenBank under accession numbers EU082134–EU082176.

Only 11 (25%) of the 43 CF sputum culture isolates were identified correctly as *Bordetella* spp. by the referring laboratory, with the remainder being identified as *Achromobacter* spp. (n = 11), *Burkholderia* spp. (n = 2), or *Pseudomonas* spp. (n = 1), or as 'non-lactose-fermenting Gram-negative rod' or 'unidentified' (n = 18). A variety of commercial phenotypic test systems were used by the referring laboratories (Table 1).

The 43 isolates were analysed at the BcRLR by using a range of species-specific 16S rDNA PCR assays for CF-related bacterial species, including species of *Pseudomonas*, *Stenotrophomonas*, *Burkholderia*, *Ralstonia*, *Pandoraea* and *Achromobacter* [2,3,5–11]. All PCR assays were negative, except that 33 (77%) of the 43 isolates were positive according to a PCR assay designed to be specific for *A. xylosoxidans* [3]. However, cluster analysis of DNA profiles generated by BOX-PCR placed these 33 isolates (as well as the remaining ten isolates) in a cluster distinct from that formed by all other *Achromobacter* strains (including reference strains) in the BcRLR's collection (results not shown). 16S rDNA sequence analysis of the 43 isolates revealed that 23 were *B. bronchiseptica/parapertussis*, five were *B. hinzii*, four were *Bordetella petrii*, three were *B. avium*, and eight were unidentified *Bordetella* spp.

Although *Bordetella* spp. are not normally considered when evaluating CF sputum cultures, occasional case reports have described the recovery of some species, including *B. hinzii* [12,13], *B. bronchiseptica* [14,15] and *B. petrii* [16], from individual CF patients. To our knowledge, *B. avium* has not been identified previously in CF sputum cultures.

The frequency with which these species are involved in pulmonary infection in CF patients is unclear. During the 6-year period of this study, sputum isolates were analysed from 874 CF patients (receiving care in 183 treatment centres), with *Bordetella* strains isolated from 43 (5%) patients. Although this rate is comparable to that of some other CF pathogens, including *Burkholderia* spp., there was a potential bias in the sample set in that care centres are more likely to refer 'atypical' isolates for which the species identification may be in question. The presence of other, more common, CF-related pathogens, including *Achromobacter*, *Burkholderia*, *Pseudomonas* and *Stenotrophomonas*, was detected in only 13 (30%) of the 43 patients. This raises the possibility that the recovery of *Bordetella* may be influenced by the presence of other species that might ordinarily overgrow *Bordetella* in culture. If this is the case,

Table 1. Putative species identifications and phenotypic test systems used by referring laboratories for 43 isolates of *Bordetella* spp.

	Phenotypic test system						
Identification	**Microscan[a]**	**Rapid NF[b]**	**Vitek[c]**	**API NF[c]**	**PASCO[d]**	**Biochem[e]**	**N/A[f]**
Bordetella spp. (n = 11)[f]	3	2	3	1	1		
Achromobacter spp. (n = 11)	3	3	1			2	2
Burkholderia spp. (n = 2)	1				1		
Pseudomonas spp. (n = 1)							1
NLFGNR (n = 18)	2	2	6	1	1	3	3

N/A, method not specified; NLFGNR, non-lactose-fermenting Gram-negative rod.
[a]Dade Behring Inc., West Sacramento, CA, USA.
[b]Remel Inc., Norcross, GA, USA.
[c]bioMérieux, Durham, NC, USA.
[d]PASCO Laboratories, Wheat Ridge, CO, USA.
[e]Short biochemical battery.
[f]One isolate was identified by the referring laboratory as *Bordetella* by 16S rDNA sequence analysis.

the prevalence of *Bordetella* within the CF population may well be underestimated. However, a prospective study of a non-biased sample set would be necessary to reach firm conclusions in this respect.

The role that these species may play in the progression of CF pulmonary disease is also unclear. *Bordetella*-infected patients ranged in age from 3 to 53 years, with 25 (58%) being aged <18 years. Only a single *Bordetella* isolate was identified among sputum culture isolates obtained serially for 41 of the 43 patients, suggesting transient colonisation with *Bordetella*. However, chronic infection is possible, with serial cultures from two patients yielding the same *Bordetella* strain for periods of 2 years (*B. petrii*) and 5 years (unidentified *Bordetella* sp.), respectively. No data were available concerning antimicrobial therapy given to these two patients during these periods.

The analysis revealed a high rate of misidentification of *Bordetella* spp. according to commercially available microbial identification systems, with 75% of the isolates (from 11 centres) being misidentified by the referring laboratory (Table 1). Misidentification of *Bordetella* as *Achromobacter* was particularly common, perhaps because of the incomplete and changing taxonomy of these two phylogenetically closely related genera. Indeed, a PCR assay designed to be specific for *A. xylosoxidans* on the basis of then available 16S rDNA sequence data [3] was positive for several isolates that were identified subsequently as *Bordetella* spp. It is noteworthy that cluster analysis of 16S rDNA sequences of the eight isolates identified as 'unclassified *Bordetella*' suggested the presence of at least four distinct, potentially novel, *Bordetella* spp. (data not shown).

In summary, this report describes the largest set of CF patients from whom *Bordetella* spp. have been identified, including the probable first report of *B. avium* infection in this patient population. Most isolates were identified incorrectly in initial testing by the referring laboratories, suggesting that further consideration should be given to the possible presence of *Bordetella* spp. in the evaluation of CF sputum culture.

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