Original Article

Bordetella bronchiseptica in a pediatric Cystic Fibrosis center☆

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7 May 2013; 2 August 2013; 5 August 2013
Available online 4 September 2013

Abstract

Background: Bordetella bronchiseptica is a common pathogenic or colonizing organism of domestic mammals. In dogs, it causes an infectious tracheobronchitis known as Kennel Cough. Human infections are unusual and almost exclusively described in immunocompromised patients who have had contact with a known animal reservoir. It is rarely reported in Cystic Fibrosis (CF), possibly hampered by low recovery from culture and organism misidentification. We describe the incidence and characteristics of B. bronchiseptica in our CF population.

Methods: A retrospective cohort study was conducted of our center’s CF patient population. Patients were included if they had B. bronchiseptica isolated on one or more occasion.

Results: Seven children with CF isolated B. bronchiseptica on 23 occasions, frequently associated with the symptoms of a pulmonary exacerbation. Four patients required hospitalization.

Conclusion: These results suggest that B. bronchiseptica may be more common than previously reported and may play a potential pathogenic role in CF.

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Keywords: Bordetella bronchiseptica; Pathogen; Animal; Cystic Fibrosis

1. Introduction

Bordetella bronchiseptica is a gram-negative medium sized rod, that was first described by Ferry [1] in 1910 as the veterinary pathogen responsible for respiratory disease in a wide range of mammals including dogs, horses, rabbits, swine, rodents and cats. In dogs, it causes an infectious tracheobronchitis commonly known as “kennel cough”. B. bronchiseptica is a major cause of snuffles in rabbits, pneumonia in cats, and atrophic rhinitis in piglets [1,2]. It has been identified as a respiratory pathogen in horses [3]. Infection with this organism can lead to chronic and asymptomatic colonization in animals which may persist in the upper respiratory tract indefinitely [4,5]. B. bronchiseptica is rarely associated with human disease but has been reported as a cause of pertussis-like disease [2]. B. bronchiseptica and Bordetella pertussis are closely related species that exhibit little genetic variation [2,6]. B. pertussis, the causative agent of whooping cough, is strictly a human pathogen. Analysis of the genomic sequences indicates that B. pertussis evolved from a B. bronchiseptica-like ancestor as a human-adapted lineage. Human adaptation appears to be a consequence of gene deletions and the disease severity is related to loss of gene regulatory functions [6]. Both strains have virulence factors in common which are regulated by the complex Bordetella virulence gene (BvgAS). BvgAS is a two-component signal transduction system which responds to certain environmental conditions as Bordetella travels within and between mammalian hosts [7]. Except for the pertussis toxin, B. bronchiseptica produces nearly all the factors associated with B. pertussis virulence. Expressed virulence factors exist in three different phases: a virulent Bvg+ phase, a non-virulent Bvg− phase and an intermediate Bvgi phase that is
associated with little to no disease. The Bvg+ phase is necessary for respiratory tract colonization characterized by the expression of adhesions, autotransporters and toxins. Adhesions and autotransporters, such as filamentous hemagglutinin (FHA), fimbriae, and pertactin, help the organisms adhere to the cilia of respiratory epithelial cells resulting in stasis of the cilia and difficulty clearing mucus secretions [3]. Adenylate cyclase toxin (CyaA), also expressed in the Bvg+ phase, interferes with the host immune response allowing the organisms to grow to higher numbers and persist longer in the respiratory tract [8]. Studies using phase-locked mutants have determined that the Bvg+ phase was sufficient for respiratory colonization and infection [7,9]. The non-virulent Bvg− phase is characterized by motility in B. bronchiseptica and is required for survival outside of the host in nutrient-limiting conditions suggesting an environmental reservoir [2,7]. Although never proven, the Bvg+ phase may be important in aerosol transmission of B. bronchiseptica between hosts [7,9,10].

Several studies have tried to provide an explanation for the long-term persistence of B. bronchiseptica in the mammalian host. A frequent hypothesis to explain continued persistent infection or asymptomatic carriage is that these organisms exist as biofilms [5,11]. The role of biofilm formation in pathogenesis has been linked to chronic bacterial infections such as CF lung disease caused by Pseudomonas aeruginosa, a disease that has become widely accepted as a biofilm-like infection [12]. Irie et al. investigated B. bronchiseptica virulence factors in relationship with biofilm formation, determining that the expression of FHA was required to obtain maximal biofilm formation in the Bvg+ phase in vitro and was primarily phase specific. They further showed that CyaA, when interacted with FHA in the Bvg+ phase, suppressed biofilm formation suggesting different requirements for acute versus persistent infection [5,12]. This was a slight variance from the study performed by Mishra et al., showing robust biofilm formation in both the Bvg+ and Bvg− phases [11]. Although there were different conclusions, both studies were in agreement that the BvgAS system was important in the regulation of biofilm formation [11]. Since then, Sloan et al. examined the nasal tissue from mice infected with B. bronchiseptica. They were able to demonstrate biofilms adherent to the nasal epithelium having similar characteristics to biofilms formed in vitro on abiotic surfaces. From this data, they suggested a possible role of biofilms in human infection [13,14]. Histopathology findings from explant tissue infected with B. bronchiseptica and B. pertussis and tissue biopsies of pertussis patients have shown damage to the ciliated epithelial cells with masses of extracellular bacteria in the cilia, structures suggestive of biofilms [15–17]. The pathology findings along with studies demonstrating biofilm formation in mice further support the hypothesis of biofilm mode of existence.

Despite frequent contact with domestic animals, the infection caused by B. bronchiseptica rarely has been reported in humans [18,19]. Literature review yields only case reports typically of immune-compromised patients who had contact with domestic animal(s) known to harbor B. bronchiseptica. B. bronchiseptica has been reported in patients with HIV/AIDS [20], cancer [21], transplants [22,23], and the elderly [24]. In addition, cases of B. bronchiseptica were reported in patients with other conditions including trauma [25], hemodialysis [26,27], Sickle Cell Disease and Down syndrome [28]. Cases of immune-competent patients contracting B. bronchiseptica have been reported on rare occasion [29], as have reports of persistent infections [30].

Reports of B. bronchiseptica in CF are extremely rare. Ner et al. reported four cases in CF patients, two of which were recent lung transplant recipients, and presumed to be immune compromised [31]. In their control population of 532 CF patients, only two cases were found (0.04%). Wallet et al. [32] reported a 27-year-old woman with CF who, after isolation of B. bronchiseptica from sputum, suffered a major decline in lung function over two years. In Wernli’s 15 year retrospective review of B. bronchiseptica isolates from a single institution, 2 of 8 isolates were from patients with CF [19]. Register et al. described isolation of B. bronchiseptica in an 11 year old patient with CF who had domestic exposure to a kitten with an acute respiratory illness. Genetic characterization of the organism implied feline to human transmission [33]. The Burkholderia cepacia Research Laboratory and Repository (BeRRLR) evaluated 874 CF respiratory tract isolates, identifying Bordetella spp. (5%) from 43 patients [34]. Based on 16S ribosomal DNA sequence data, 23 Bordetella spp. isolates were identified as B. bronchiseptica/parapertussis. All isolates had been assessed using a phenotypic commercial identification system prior to submission by the referring laboratories. BeRRLR analysis of the 43 Bordetella isolates revealed that only 25% (n = 11) were correctly identified by the referring laboratories with the remainder misidentified or unidentified [34,35].

Overgrowth by more common CF pathogens including mucoid P. aeruginosa, may conceal lower numbers of B. bronchiseptica making isolation technologically challenging. We hypothesize that B. bronchiseptica may be more common than previously described in patients with CF due to difficulties with isolation and accurate identification. In addition, we postulate that B. bronchiseptica may be associated with or without the symptoms of a pulmonary exacerbation in patients with CF. The aim of this study is to describe the frequency of B. bronchiseptica isolation, the circumstances, the symptoms associated with isolation of B. bronchiseptica, and the antimicrobial sensitivities of the organism in our CF center’s patient population.

2. Methods

2.1. Study design

A retrospective review was conducted of the Children’s Hospitals and Clinics of Minnesota’s CF center between 1991 and 2012 following approval from our Institutional Review Board (IRB). Patients were included if they had a positive airway culture for B. bronchiseptica from the hospital laboratory, and a diagnosis of Cystic Fibrosis confirmed by sweat chloride values >60 mEq/L measured by quantitative pilocarpine iontophoresis and/or genetic testing positive for 2 known CF-causing mutations. The study period was defined as
one year before the first isolate of *B. bronchiseptica* and one year after the last isolate of *Bordetella*. Records from the Children’s Hospitals and Clinics of Minnesota and Children’s Respiratory and Critical Care Specialists were reviewed for demographics, diagnoses, lung function, nutritional status, and exposure to domestic animals. In addition, data were collected for previous or concurrent airway cultures, sensitivities, signs and symptoms of pulmonary exacerbations, hospitalizations, treatment, and response to therapy. Fuchs [36] criteria was applied to identify signs and symptoms of pulmonary exacerbations including decline in FEV1, new onset cough, shortness of breath, sputum production, wheezing, crackles, chest x-ray changes, and weight loss. For the purpose of this study, the baseline FEV1 was determined as the best FEV1% predicted within one year prior to the isolation of *B. bronchiseptica*.

### 2.2. Statistical methods

Descriptive statistics were used to describe the characteristics of the study population. Quantitative variables are expressed as mean, median and range. To estimate prevalence during the study periods, the registry data from PortCF and the Epidemiological Study of Cystic Fibrosis (ESCF) were reviewed to quantify the number of active patients with CF that were receiving care at our institution.

### 2.3. Laboratory isolation and identification

Respiratory specimens were submitted to the laboratory for patients with CF during routine or episodic encounters. The samples were plated on a combination of selective and non-selective agar plates and incubated according to the recommendations taken from the Cystic Fibrosis Foundation consensus report [37–39]. *B. bronchiseptica* is a glucose non-fermentative rod which has simple nutritional requirements, growing in 1–2 days on common laboratory media such as 5% sheep blood and MacConkey agar. Phenotypic identification was performed using a commercial biochemical system, Vitek (bioMérieux), on all suspicious isolates including conventional biochemicals when necessary. Vitek was unable to identify one isolate, which was subsequently misidentified as *Cupriavidus pauculus* (formerly CDC group IVC-2) by Siemens MicroScan due to a similar phenotypic profile.

### 3. Results

Available registry data during the study period reflected a growing CF center. The pediatric CF center had an ESCF enrollment of 59 patients in 2002 and 137 patients enrolled in PortCF in 2012. Seven patients with a median age of 12 years (range: 1–20) with CF had *B. bronchiseptica* isolated in airway cultures on 23 occasions. Four patients grew *B. bronchiseptica* once, and three on multiple occasions. All patients had at least one copy of the most common CF causing mutation, ΔF508, and 5 were homozygous for ΔF508. All 7 patients had documented exposure to domestic animals known to harbor *B. bronchiseptica* (Table 1) and the majority of patients 4/7 (57%) either lived on a farm or operated a kennel on the home property (Table 1). None of our patients were transplant recipients and none had any other diagnosis known to impart immune deficiency. All patients had mild CF lung disease at baseline. Of those able to perform routine spirometry, the median FEV1 was 99% predicted (range 79–121% predicted). Based on the study range of PortCF registrants, the estimated prevalence in our population could range from 5 to 12%.

#### 3.1. Signs and symptoms at time of initial *B. bronchiseptica* isolation

All patients experienced an increased cough and 86% (6/7) had additional signs and symptoms of a pulmonary exacerbation such as decrease in FEV1 and chest x-ray changes when *B. bronchiseptica* was first isolated from airway cultures (Table 2). One patient exhibited a pattern of cough different from previous pulmonary exacerbations of CF, which was clinically similar to *B. pertussis* infection. Two patients were exposed to animals with a veterinarian-diagnosed case of “kennel cough”.

#### 3.2. Intercurrent microorganisms

All patients had positive cultures for methicillin-sensitive *Staphylococcus aureus* (MSSA) within the year preceding, or concurrent with the time of *B. bronchiseptica* isolation. One patient had intermittent colonization of methicillin-resistant *S. aureus* (MRSA). None of our patients had historic isolation of *P. aeruginosa* (mucoid) from airway cultures. Three patients isolated *P. aeruginosa* (matte) intermittently.

#### 3.3. Antibiotic sensitivity

Fifteen of the 23 *B. bronchiseptica* isolates had antibiotic sensitivities reported by the lab. Although antibiotic sensitivities and treatment for *B. bronchiseptica* infection varied, most isolates were resistant to common beta lactam antibiotics (Table 3).

#### 3.4. Patient outcomes

All symptomatic patients were treated with antibiotics based on available sensitivity data. Piperacillin/tazobactam, trimethoprim/sulfa, ciprofloxacin, and meropenem were the most frequently used antibiotics in our patient population. Patient #4, who had *B. bronchiseptica* isolated on 13 occasions, has a sulfa allergy (Table 3).

Four patients were hospitalized for pulmonary exacerbations coincident with *B. bronchiseptica* isolation. *B. bronchiseptica* and MSSA were present concomitantly in airway cultures in all four patients. These patients had historically grown MSSA while asymptomatic and continued to have positive cultures for MSSA following treatment of *B. bronchiseptica* and resolution of symptoms. While patients responded favorably to treatment directed at *B. bronchiseptica*, four patients had recurrence and...
one patient had *B. bronchiseptica* isolated on 13 occasions. We have recommended vaccination of the pets but this has not been accomplished. Three of our patients lived on small farms and had exposure to multiple animal reservoirs. One patient’s family operated a kennel.

4. Discussion

In our CF center, the majority of *B. bronchiseptica* isolates have been associated with the CF population. All patients had contact with a known domestic animal reservoir. Those who live in rural environments with exposure to multiple animals may be at more risk. We speculate that the CF lung with its impaired host defense including impaired mucus clearance, and ciliary dysfunction may selectively predispose CF patients to *B. bronchiseptica*. In addition, our experience suggests that the incidence of *B. bronchiseptica* while rare, is higher than previously reported in non-transplant patients with CF and approximates Ner’s observations [31,35]. Because laboratory isolation and identification of CF microbes can be technically challenging and difficult, *B. bronchiseptica* may be underreported or misidentified. In our experience, the diligence by our hospital lab resulted in treatment decisions that had a direct patient benefit.

While the significance of *B. bronchiseptica* in CF is unclear, our findings suggest that it may act as both a colonizer and pathogen. All patients were symptomatic at the initial isolation of *B. bronchiseptica*. Although all of our patients had concurrent growth of MSSA, they also had positive MSSA cultures when asymptomatic (Table 3). Some patients had persistent colonization of MSSA following treatment and resolution of symptoms when *B. bronchiseptica* did not recur. This suggests that treatment of *B. bronchiseptica* resulted in the resolution of symptoms. On multiple occasions *B. bronchiseptica* was isolated from one patient, even when asymptomatic (Table 3). This suggests that *B. bronchiseptica* may also act as a colonizer. The potential of biofilm formation in *B. bronchiseptica* may explain the mechanism by which *B. bronchiseptica* was observed as a colonizer.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Genotype</th>
<th>Sweat chloride mEq/L</th>
<th>Gender</th>
<th>Age (years) at first isolate</th>
<th>Number of isolate events</th>
<th>Baseline FEV1 % predicted</th>
<th>Concurrent airway isolates</th>
<th>Animal contact</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>DeltaF508/DeltaF508</td>
<td>84/84</td>
<td>F</td>
<td>1.9</td>
<td>2</td>
<td>N/A</td>
<td>MSSA, <em>B. bronchiseptica</em></td>
<td>Dog</td>
</tr>
<tr>
<td>2</td>
<td>DeltaF508/DeltaF508</td>
<td>103/111</td>
<td>M</td>
<td>4</td>
<td>1</td>
<td>N/A</td>
<td><em>B. bronchiseptica</em>, MSSA, <em>P. aeruginosa</em> (matte)</td>
<td>Dogs, horses, cats, guinea pigs/farm</td>
</tr>
<tr>
<td>3</td>
<td>DeltaF508/DeltaF508</td>
<td>N/A</td>
<td>F</td>
<td>8</td>
<td>1</td>
<td>87</td>
<td><em>B. bronchiseptica</em>, MSSA, group A streptococci, <em>Stenotrophomonas maltophilia</em></td>
<td>Dogs, cats</td>
</tr>
<tr>
<td>4</td>
<td>DeltaF508/Delta 1507</td>
<td>113</td>
<td>F</td>
<td>12</td>
<td>14</td>
<td>98</td>
<td><em>B. bronchiseptica</em> (previously misidentified as <em>Cupriavidus pauculus</em>), MSSA</td>
<td>Dog with “kennel cough”, horses/small farm</td>
</tr>
<tr>
<td>5</td>
<td>DeltaF508/DeltaF508</td>
<td>108</td>
<td>F</td>
<td>13</td>
<td>1</td>
<td>79</td>
<td><em>B. bronchiseptica</em>, <em>P. aeruginosa</em> (matte), MSSA</td>
<td>Dogs, cats</td>
</tr>
<tr>
<td>6</td>
<td>DeltaF508/unknown</td>
<td>90</td>
<td>F</td>
<td>14</td>
<td>2</td>
<td>121</td>
<td><em>B. bronchiseptica</em>, MSSA, yeast</td>
<td>Dogs (ill)/kennel business</td>
</tr>
<tr>
<td>7</td>
<td>DeltaF508/DeltaF508</td>
<td>N/A</td>
<td>M</td>
<td>20</td>
<td>2</td>
<td>112</td>
<td><em>B. bronchiseptica</em>, MSSA, yeast</td>
<td>Dog/farm</td>
</tr>
</tbody>
</table>

*Best FEV1% Predicted value one year prior to first *B. bronchiseptica* culture.*

*Patient too young to perform PFTs.*

*Data not available from institutional record.*
Table 3
Patient outcomes after first \textit{B. bronchiseptica} isolate.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Antibiotic treatment</th>
<th>Baseline pre-treatment FEV$_1$% predicted</th>
<th>Post treatment FEV$_1$% predicted</th>
<th>Hospitalization</th>
<th>Pre-treatment culture</th>
<th>Post treatment culture</th>
<th>Number of subsequent isolates</th>
<th>Number of subsequent isolates when asymptomatic</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Azithromycin</td>
<td>N/A $^b$</td>
<td>N/A $^b$</td>
<td>No</td>
<td>MSSA, \textit{B. bronchiseptica}, Moraxella catarrhalis</td>
<td>MSSA</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>Cipro</td>
<td>N/A $^b$</td>
<td>N/A $^b$</td>
<td>No</td>
<td>\textit{B. bronchiseptica}, \textit{MSSA}, \textit{P. aeruginosa} (matte)</td>
<td>\textit{P. aeruginosa}</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>Trimethoprim/\text{ sulfamethoxazole}</td>
<td>87</td>
<td>92</td>
<td>No</td>
<td>\textit{B. bronchiseptica}, \textit{MSSA}, group A streptococci, \textit{Stenotrophomonas maltophilia}</td>
<td>\textit{MSSA}</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>Meropenem Tobramycin</td>
<td>98</td>
<td>97</td>
<td>Yes</td>
<td>\textit{B. bronchiseptica}, \textit{MSSA}</td>
<td>\textit{MSSA}</td>
<td>13</td>
<td>4</td>
</tr>
<tr>
<td>5</td>
<td>Ceftazadime Tobramycin/\text{TMP/SMX}</td>
<td>79</td>
<td>75</td>
<td>Yes</td>
<td>\textit{B. bronchiseptica}, \textit{MSSA}, \textit{P. aeruginosa} (matte), \textit{MSSA}</td>
<td>\textit{MSSA, Trichosporon species}</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>Clindamycin Ceftazadime</td>
<td>121</td>
<td>129</td>
<td>Yes</td>
<td>\textit{B. bronchiseptica}, \textit{MSSA, yeast}</td>
<td>\textit{Staphylococcus sp., Stomatococcus sp., alpha-hemolytic streptococci}</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>Clindamycin Ceftazadime/Tobramycin Itraconazole</td>
<td>112 $^a$</td>
<td>115</td>
<td>Yes</td>
<td>\textit{B. bronchiseptica}, \textit{MSSA, yeast}</td>
<td>\textit{MSSA, yeast}</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

$^a$ Best FEV$_1$% predicted value one year prior and post first \textit{B. bronchiseptica} culture.

$^b$ Patient too young to perform PFTs.

Chromatically colonizing pathogen and the unsuccessful attempts at eradication. In addition this patient has continued exposure to unimmunized horses in a boarding environment raising the likelihood of reinfection.

The pathogenicity of \textit{B. bronchiseptica} in the CF lung and the significance of isolation especially as an emerging pathogen need further examination. As successful therapies targeting \textit{P. aeruginosa} improve the care of CF patients and increase the life spans, other potential pathogens have emerged. Organisms such as \textit{Stenotrophomonas maltophilia}, \textit{Alcaligenes xylosoxidans}, \textit{Ralstonia sp.}, \textit{Burkholderia gladioli} and other \textit{Burkholderia spp.}, \textit{Inquilinus limosus} and \textit{Pandoraea} spp. are increasing in frequency among CF patients [37]. We recommend the close scrutiny of unusual isolates found in CF airway cultures particularly gram-negative organisms. Correct identification of these isolates has value in assessing the clinical importance of therapies that may benefit the CF patient. Recent advances in molecular methods have provided better strategies for accurate identification of unusual CF strains and have become a useful tool when phenotypic identification fails or is doubtful [34,35,39,40].

Although commercial phenotypic identification systems have been validated for the identification of non-fermentative gram-negative rods, the accuracy is limited or uncertain when used to identify atypical CF strain [34,39,35]. CF strains often produce colonies that lack key metabolic characteristics and have biochemical variability that can cause misidentifications when using commercial identification systems. More recently, strategies using a combination of conventional biochemical tests and molecular methods (e.g., 16S rRNA sequencing and MALDI-TOF) for identification of \textit{B. bronchiseptica} and other difficult-to-identify CF organisms have allowed for more rapid and reliable identification results. While the 16S rRNA gene has been a common target for single pathogen identification, researchers have now begun to characterize the complete CF microbial community using next generation sequencing targeting the same gene [41]. These studies have the potential to provide new information regarding the pathogenic microbiomes in CF.

While the role of \textit{B. bronchiseptica} in CF lung disease remains unclear, the ability to inhibit leukocyte function, interfere with mucus clearance, and the potential to form biofilms that contribute to colonization and persistence in the lower respiratory tract may explain our observations of \textit{B. bronchiseptica} as a possible pathogen and colonizer. We encourage routine environmental histories of all CF patients including the presence of pets, or domestic farm animals, and \textit{B. bronchiseptica} immunization status.

We recommend routine \textit{B. bronchiseptica} immunization of all domestic mammalian pets and farm animals to reduce the reservoir for this potential pathogen.

Acknowledgments

We thank Christopher McNamara and Ashley Young for their additional support in the historical data collection.