Microbial Cultures of the Respiratory Tract in Familial Dysautonomia Lung Disease

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Abstract

Objective: To determine the microbial pathogens in the lungs of patients with Familial Dysautonomia (FD).

Methods: Medical records of 250 FD patients were retrospectively evaluated. Respiratory tract culture results retrieved by Bronchoalveolar Lavage (BAL) or Deep Bronchial Lavage (DBL), deep sputum, or Endotracheal Aspirate (ETA), were reviewed. The percentages of the positive and negative results for each patient were calculated, and the Confidence Intervals (CI) were generated using Winpepi software.

Results: A total of 266 cultures, 125 from BAL-DBL, 67 via sputum, and 74 via ETA were analyzed. Twenty six species of bacteria and ten species of fungi were identified. Out of the 97 patients, 55.7% (54 patients) had at least one positive culture. The most common overall bacterial pathogen was Haemophilus influenzae (32.0%), while the most common fungal pathogen was Candida albicans (11.3%). The most common bacterial pathogen from BAL-DBL and deep sputum was Haemophilus influenza (23.2% and 37.9% respectively), and via ETA it was Pseudomonas aeruginosa (80%). The most common fungal pathogen by BAL-DBL was Candida albicans (8.5%), by deep sputum no particular fungi were prevalent, and via ETA the only fungal pathogen was Candida albicans (40%).

Conclusions: The most common pathogen both overall and from BAL-DBL and deep sputum retrieval was Haemophilus influenzae, while the most common pathogen via ETA was Pseudomonas aeruginosa. The prevalence of each microbial species differed based on the method that the culture was obtained.

Keywords: Aspiration; Pneumonia; Bronchoalveolar Lavage; Endotracheal Aspirate; Familial Dysautonomia

Abbreviations: BAL: Bronchoalveolar Lavage; CAP: Community Acquired Pneumonia; CI: Confidence Intervals; COPD: Chronic Obstructive Pulmonary Disease; CF: Cystic Fibrosis; DBL: Deep Bronchial Lavage; ETA: Endotracheal Aspirate; FD: Familial Dysautonomia; GER: Gastroesophageal Reflux; LFP: Low-power Field; PMN: Polymorphonuclear Neutrophils; SECs: Squamous Epithelial Cells; TTA: Transtracheal Aspiration; VAP: Ventilator Associated Pneumonia.

Introduction and Background

Familial Dysautonomia (FD), also known as Riley-Day Syndrome, and Hereditary Sensory and Autonomic Neuropathy type III (HSAN III), is an autosomal recessive disease mostly affecting people of Ashkenazi Jewish descent [1]. Patients with FD have abnormal development of autonomic and sensory nervous system, resulting in malfunction of most body systems, such as the gastrointestinal, respiratory, cardiovascular, orthopedic, renal and neurological systems.

Chronic lung disease is the most common cause of morbidity and mortality in the FD patient population [1]. Forty four percent of FD patients died of pneumonia [1]. The main reason is pulmonary aspiration caused by defective coordination of the nasopharynx, cricopharyngeal and esophageal muscles, and malfunction of the gastroesophageal junction resulting in Gastroesophageal Reflux (GER) [1]. Poor sucking reflex in infancy, increased salivation [1] and recurrent bouts of vomiting also contribute to the aspiration [2]. These deficiencies can cause fluid, food and saliva to be aspirated into the lungs resulting in recurrent bronchopneumonia and chronic lung disease [2]. It is well known that patients with cystic fibrosis (CF) have a higher incidence of pulmonary infections due to Pseudomonas aeruginosa [3]. In addition, there have been studies showing that Haemophilus influenzae is most frequently seen in patients with chronic obstructive pulmonary disease (COPD) [3], and other studies showed that both adults and children with aspiration pneumonia in debilitating conditions, tracheal-esophageal malformations, central nervous system disease, and altered consciousness, are prone to infection by anaerobic bacteria [4,5]. However, there have not been any studies on the microbiota isolated associated with pulmonary infections in FD patients. Therefore, this study sought to determine the frequencies of potential respiratory pathogens in FD patients, in order to provide evidence base for the selection of microbiological bacteria. That will allow applying empiric therapy for this specific patient population while microbiological results are still pending. In addition, FD might serve as a model for other diseases associated with lung aspiration, such as cerebral palsy and neurological and muscular disorders.

Materials and Methods

Study Design

This retrospective study was designed to investigate the microbial species isolated from the respiratory tracts of FD patients with pulmonary infections. The study was conducted at Israeli Center for Familial Dysautonomia, Hadassah-Hebrew University Medical Center, in Mount Scopus, between 1987 and 2012. Data were obtained directly from the charts of 250 FD patients. Patients included in the study had a diagnosis of FD confirmed by genetic testing, a histamine test, and clinical signs and symptoms. Both living and deceased patients were included in this study. The study received an IRB approval (0505-12-HMO) and informed consent was waived.

Generation of Data

Out of the 250 patient files studied, 97 had at least one documented respiratory tract microbial culture. All respiratory
cultures were taken for diagnostic purposes. Data collected included the date, mode of collection, culture findings, and age of patient at the time of retrieval.

Respiratory Cultures

Respiratory samples were collected in each patient following three days without antibiotic treatment by at least one of three methods:

1. Bronchoalveolar Lavage or Deep Bronchial Lavage (BAL, DBL) were performed under aseptic conditions in an operating room or the Intensive Care Unit in FD patients who needed bronchoscopy for lavage, or in FD patients with chronic lung disease who had general anesthesia for various scheduled operations. A 3.6 mm fiber-optic flexible bronchoscope or a long thin flexible catheter was inserted until the bronchoscope or catheter reached the desired location in the lung, according to the clinical or radiological findings. Sterile saline solution was introduced, and the culture was collected by suction through the bronchoscope or catheter.

2. Deep coughed sputum was expectorated into a sterile cup, usually following chest physiotherapy.

3. Endotracheal Aspirates (ETA) were collected using a short flexible catheter inserted by a nurse or physiotherapist through a tracheostomy or through the endotracheal tube in ventilated patients.

Laboratory Procedures

All specimens from the respiratory tract were screened for oropharyngeal contamination by examination of Gram stained films, by a procedure recommended by the American Society for Microbiology [6]. The criteria used for expectorated sputum and ETA was as follows: the presence of > 10 Squamous Epithelial Cells (SECs) per low-power field (lpf, X100) resulted in rejection of a specimen for culture. In the absence of SECs, specimens with > 25 Polymorphonuclear neutrophils (PMNs) per lpf were accepted for culture. For BAL and DBL, in which oropharyngeal contamination may occur during introduction of the bronchoscope or catheter, and in which there is a significant dilution factor in obtaining the specimen, the criteria were different: the presence of PMNs was not a requirement, but the presence of any SECs resulted in rejection for culture. Specimens were cultured on 5% sheep blood agar, chocolate agar and MacConkey agar (Novamed, Jerusalem). Organisms isolated were identified by standard classical biochemical and physiological tests [7]. It should be noted that diagnostic techniques improved with the advent of various commercial identification systems (e.g. the API series of identification kits, BioMerieux, Marcy l’Etoile, France). Anaerobic culture of these specimens is not performed. Primary isolation for fungi, when requested, was inoculation of Sabouraud dextrose agar (Novamed, Jerusalem). Organisms isolated were identified by standard classical biochemical and physiological tests [7]. It should be noted that diagnostic techniques improved with the advent of various commercial identification systems (e.g. the API series of identification kits, BioMerieux, Marcy l’Etoile, France).

Statistical Methods

The percentages of the positive and negative results for each patient were calculated, and the Confidence Intervals (CI) were generated using Winpepi software (CI of 95% was used). Regardless of the number of specimen results the patient had, a duplicate result was only counted once (first culture) for each individual patient.

Results

Of the 250 FD patient charts, 97 had at least one respiratory culture documented and were included in the study. Forty six patients were female and fifty one male. The age that the specimen cultures were taken ranged from three months to 53 years old, mean 12.4 years, SD 9.9.

A total of 266 cultures, 125 via BAL-DBL (105 via catheter-DBL, 20 via bronchoscopy- BAL), 67 via sputum, and 74 via ETA were analyzed. A total of 26 species of bacteria, and 10 species of fungus were found. Out of the 97 patients, at least one positive culture was found in 55.7% (54 patients) via approach two (all), 54.9% (45 patients) via BAL, 75.9% (22 patients) by deep sputum test, and 100% (10 patients) via ETA. At least one culture with mixed organisms was found in 19.6% via approach two (all specimens), 18.3% via BAL-DBL, 13.8% testing deep sputum, and 10.0% via ETA.

Using approach two (table 1), the most common bacterial pathogen obtained overall was Haemophilus influenzae (32.0%, CI = 22.85 - 42.20%), followed by Pseudomonas aeruginosa (19.6%, CI = 12.22 - 28.89%) and Streptococcus pneumonia (12.37% CI = 6.56 - 20.61%).

Using approach one, the most common bacterial pathogen via BAL was Haemophilus influenzae (23.2 %, CI = 14.56 - 33.80 %), followed by Pseudomonas aeruginosa (24.1 % CI = 10.30 - 43.54%) and Streptococcus pneumonia (13.8 % CI = 3.89 - 31.66%).

The most common bacterial pathogen by deep sputum was Haemophilus influenzae (37.9% CI = 20.69 - 57.74%), followed by Pseudomonas aeruginosa (24.1% CI = 10.30 - 43.54%) and Streptococcus pneumonia (13.8 % CI = 3.89 - 31.66%).

The most common bacterial pathogen via ETA was Pseudomonas aeruginosa (80%, CI = 44.39 - 97.48%) followed by Staphylococcus aureus (50%, CI = 18.71 - 81.29%) and Haemophilus influenzae (40%, CI = 12.16 - 73.76%).

Out of the 97 patients, 15 patients had at least one of 10 different species of fungus. Candida albicans was the most common fungus, found in 73.3% of patients with a positive fungus culture.

Using approach two (table 1), the most common fungus obtained overall was Candida albicans (11.3% CI = 5.80 - 19.39%), followed by Candida tropicalis (3.1%, CI = 0.64 - 8.77%) and Candida parapsilosis (2.1%, CI = 0.25 - 7.25%). Using approach one, the most common fungal pathogen via BAL was Candida albicans (8.5%, CI = 3.50 - 16.80%), followed by Candida tropicalis (3.7%, CI = 0.76 - 10.32%), Candida dubliniensis, Candida kruzei, and Candida parapsilosis (1.2% CI = 0.03 - 6.61%). Seven different species of fungus were found testing deep sputum; Candida albicans, Candida lusitaniae, Candida parapsilosis, Cladosporium species, Penicillium species, Rhodotorulaglutinis, Candida colliculosa. There was no most common fungal pathogen, each 3.4 % CI = 0.09 - 17.76 %.

The only fungus from ETA was Candida albicans (40%, CI = 12.16 - 73.76%).

The spectrum of organisms isolated is demonstrated in Table 2 and Figures 1-4. Out of the 125 cultures retrieved trough BAL-
Table 1: Most common pathogens via approach 1 (based on the method of retrieval) and 2 (all, regardless of the method of retrieval).

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>BAL-DBL</th>
<th>Sputum</th>
<th>ETA</th>
<th>All</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Acinetobacter baumannii</em></td>
<td>3 (3.7%)</td>
<td>1 (3.4%)</td>
<td>3 (30%)</td>
<td>6 (6.2%)</td>
</tr>
<tr>
<td><em>Burkholderia cepacia</em></td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>1 (10%)</td>
<td>1 (1.03%)</td>
</tr>
<tr>
<td><em>Campylobacter fetus</em></td>
<td>1 (1.2%)</td>
<td>0.03 - 6.61%</td>
<td>0 (0%)</td>
<td>1 (1.03%)</td>
</tr>
<tr>
<td><em>Citrobacter freundii</em></td>
<td>0 (0%)</td>
<td>1 (3.4%)</td>
<td>0 (0%)</td>
<td>1 (1.03%)</td>
</tr>
<tr>
<td><em>Corynebacterium glutinis</em></td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>1 (10%)</td>
<td>1 (1.03%)</td>
</tr>
<tr>
<td><em>Corynebacterium striatum</em></td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>1 (10%)</td>
<td>1 (1.03%)</td>
</tr>
<tr>
<td><em>Enteroacter aerogenes</em></td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>1 (10%)</td>
<td>1 (1.03%)</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>1 (1.2%)</td>
<td>0.03 - 6.61%</td>
<td>0 (0%)</td>
<td>1 (1.03%)</td>
</tr>
<tr>
<td><em>Haemophilus influenzae</em></td>
<td>19 (23.2%)</td>
<td>14.56 - 33.80%</td>
<td>11 (37.9%)</td>
<td>12.22 - 28.89%</td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae</em></td>
<td>1 (1.2%)</td>
<td>0.03 - 6.61%</td>
<td>0.09 - 17.76%</td>
<td>2 (20%)</td>
</tr>
<tr>
<td><em>Klebsiella oxytoca</em></td>
<td>0 (0%)</td>
<td>1 (3.4%)</td>
<td>0.09 - 17.76%</td>
<td>0 (0%)</td>
</tr>
<tr>
<td><em>Moraxella catarrhalis</em></td>
<td>6 (7.3%)</td>
<td>2.73 - 15.25%</td>
<td>3 (10.3%)</td>
<td>22.85 - 42.20%</td>
</tr>
<tr>
<td><em>Morganella morganii</em></td>
<td>0 (0%)</td>
<td>1 (3.4%)</td>
<td>0.09 - 17.76%</td>
<td>0 (0%)</td>
</tr>
<tr>
<td><em>Proteus mirabilis</em></td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>2 (20%)</td>
<td>2.52 - 55.61%</td>
</tr>
<tr>
<td><em>Provident ciarietgieri</em></td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>1 (1.03%)</td>
</tr>
<tr>
<td><em>Provident ciastuarti</em></td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>2 (20%)</td>
<td>2.52 - 55.61%</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>6 (7.3%)</td>
<td>2.73 - 15.25%</td>
<td>7 (24.1%)</td>
<td>44.39 - 97.48%</td>
</tr>
<tr>
<td><em>Serratia marcescens</em></td>
<td>0 (0%)</td>
<td>2 (6.9%)</td>
<td>0.85 - 22.77%</td>
<td>2 (20%)</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>5 (6.1%)</td>
<td>2.01 - 13.66%</td>
<td>2 (6.9%)</td>
<td>18.71 - 81.29%</td>
</tr>
<tr>
<td><em>Streptococcus CoNS</em></td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>1 (10%)</td>
<td>0.25 - 44.50%</td>
</tr>
<tr>
<td><em>Streptococcus mahlichia</em></td>
<td>0 (0%)</td>
<td>1 (3.4%)</td>
<td>0.09 - 17.76%</td>
<td>0 (0%)</td>
</tr>
<tr>
<td><em>Streptococcus anginosus</em></td>
<td>1 (1.2%)</td>
<td>0.03 - 6.61%</td>
<td>0 (0%)</td>
<td>1 (1.03%)</td>
</tr>
<tr>
<td><em>Streptococcus mitis</em></td>
<td>1 (1.2%)</td>
<td>0.03 - 6.61%</td>
<td>0 (0%)</td>
<td>1 (1.03%)</td>
</tr>
<tr>
<td><em>Streptococcus pneumoniae</em></td>
<td>7 (8.5%)</td>
<td>3.50 - 16.80%</td>
<td>4 (13.8%)</td>
<td>2 (20%)</td>
</tr>
<tr>
<td><em>Streptococcus pyogenes</em></td>
<td>0 (0%)</td>
<td>2 (6.9%)</td>
<td>0.85 - 22.77%</td>
<td>0 (0%)</td>
</tr>
<tr>
<td><em>Streptococcus viridans</em></td>
<td>3 (3.7%)</td>
<td>0.76 - 10.32%</td>
<td>0 (0%)</td>
<td>3 (3.1)</td>
</tr>
<tr>
<td><em>Streptococcus spp. undefined</em></td>
<td>1 (1.2%)</td>
<td>0.03 - 6.61%</td>
<td>0 (0%)</td>
<td>1 (1.03%)</td>
</tr>
</tbody>
</table>

Table 2: Overview of all specimen results.

DBL, 8 (6.4%) cultures showed two different isolates. 9 (13.4%) out of the 72 cultures isolated by deep coughed sputum were polymicrobial (two specimens with three different organisms, and seven specimens with two different pathogens). Lastly, 18 (24.3%) of the 74 cultures retrieved via ETA were polymicrobial (one specimen with three different isolates and 17 specimens with two). *Pseudomonas aeruginosa* was the commonest organism accompanied by additional bacteria: 54.3% of the polymicrobial specimens had *Pseudomonas aeruginosa* and 22.9% had Haemophilus influenzae.

Discussion

Until now, there have not been studies on the microbiota isolated from the lungs of FD patients. In this study we analyzed previously documented respiratory cultures of FD patients via one of three methods; BAL-DBL, deep coughed sputum, and/or ETA.
**Figure 1:** Specimen findings via BAL-DBL.

**Figure 2:** Specimen findings testing deep sputum.

**Figure 3:** Specimen findings via ETA.
A total of 26 species of bacteria and 10 species of fungus were identified. At least one culture with an infectious agent was found in 55.7% of cultures via approach two: 54.9% via BAL, 75.9% via sputum, and 100% via ETA. At least one mixed culture was found in 19.6% of all cultures via approach two, 18.3% via BAL-DBL, 13.8% testing deep sputum, and 10.0% via ETA.

One of the main causes of chronic lung disease and recurrent bronchopneumonia in FD patients are frequent pulmonary aspirations [1,2]. It has previously been shown that anaerobes play a key role in most cases of infection following aspiration [4,5]. In addition, it has been shown that *Pseudomonas aeruginosa* adherence is higher in epithelium that has been damaged by acid [2]. Lastly, it is believed that Ventilator-Associated Pneumonia (VAP) is caused by microaspiration of oropharyngeal material [8]. We therefore hypothesized that the etiology of lung infections in FD patients, specifically ventilated patients, might demonstrate anaerobes and/or *P. aeruginosa* as one of the more common organisms. However, as our results show, *H. influenzae* was found to be the most common potential pathogen obtained overall, and via BAL-DBL and deep coughed sputum. *P. aeruginosa* was the most common isolate from ETA and one of the most common from BAL-DBL and sputum retrieval.

De Schutter et al. [9] performed a retrospective study which demonstrated *H. influenzae*, *M. catarrhalis*, and *S. pneumoniae* to be the most common potential pathogens obtained via BAL in children with acute non-responding or recurrent Community Acquired Pneumonia (CAP). In another study by Avital et al. [10], *H. influenza* was also found to be the main organism obtained via BAL and oropharyngeal suction in non-cystic fibrosis patients with recurrent or persistent pneumonia. Akata et al. [11] reported that oral streptococci were the most detected bacterial phylo types in bronchoalveolar lavage fluid (BALF) samples in patients with aspiration risks. These results are similar to our results that showed *H. influenzae* to be the most common potential bacterial pathogen via BAL, followed by *S. pneumoniae*, *M. catarrhalis* and *P. aeruginosa*.

We found that the most common bacterial isolated from deep coughed sputum was *H. influenzae*, followed by *P. aeruginosa* and *S. pneumoniae*. This is slightly different than a retrospective study performed by Hoshina et al. [12], which related to children’s sputum samples. In that study [12], *H. influenzae*, *S. pneumoniae*, and *M. catarrhalis* were the most common and not *P. aeruginosa*. This discrepancy may be due to the different age of the patients. In our study, the age of the patients when the sputum cultures were retrieved ranged from three months to 53 years (mean 12.4 years, SD 9.9). Different organisms were more common at different age groups. It has been shown that *S. aureus* and *H. influenzae* were the most common pathogens in CF patients until age ten; while *P. aeruginosa* became the most common pathogen later on [13]. Our study did not consider age when looking at the prevalence of the different pathogens. In the study of Valenza [13], sputum of patients with CF, *S. aureus* was the most common, followed by *P. aeruginosa* and *H. influenzae*. These results may show that the isolates in CF patients are different than those in FD patients.

Our study showed that the most common bacteria from ETA were *P. aeruginosa*, *S. aureus*, and *H. influenzae*. These results closely reflect a previous work by Park [14], which studied the microbiology of VAP, and showed that *P. aeruginosa* was the most common, followed by *S. aureus*, Enterobacteriaceae, and Haemophilus species. According to this study, the isolates in VAP depend on patient characteristics, as well as antibiotic use, current hospitalizations, and length of time on ventilation. In another study [8], Marik et al. showed that *P. aeruginosa*, Methicillin-Sensitive *S. aureus*, Enterobacter, and *S. pneumoniae* were most common in patients with VAP. These findings are similar to our results and also demonstrate that anaerobes were not a common finding in the lungs of ventilated FD patients. Patients with VAP have a high incidence of polymicrobial infections [14]. Our results showed that 24.3% of specimens collected via ETA were polymicrobial, compared to 6.4% via BAL, and 13.4% via sputum. Additionally, *P. aeruginosa* was the most common organism to be isolated with another pathogen. Fifty four percent of the polymicrobial specimens had *P. aeruginosa*, and 22.9% had *H. influenzae*. Bacterial susceptibilities to antibiotic treatment were not part of this study as they were very diverse in the different patients and require additional study which should involve many more different parameters.

Our results via BAL-DBL were similar to results reported in other studies on non-CF children with CAP, and the results via ETA were similar to other studies of non-CF patients with VAP. However, the results via deep coughed sputum were slightly different than studies analyzing both CF and non-CF children. *H. influenzae*, *S. pneumoniae*, *P. aeruginosa*, and *M. catarrhalis* were the most common isolates in...
both BAL and deep coughed sputum, and are all common in CAP [9,12,13], while *P. aeruginosa*, *S. aureus*, and *H. influenzae* were the most common in ETA, and common in VAP [8,15].

In examining respiratory specimens, one must always consider the possibility of contamination. The upper respiratory tract contains microbiota that may be used as a defense barrier, but may also be a source for lower respiratory tract infections [15]. In a study comparing bacterial culture results of BAL and nasal lavage fluid, Wang et al. [16] found more bacterial isolates in the nasal lavage fluid then in the BAL. They show that nasal bacteriological findings are poor predictors of the actual bronchoalveolar bacteriology or that not all bacteria in the upper respiratory tract will cause an infection in the lungs. Loens et al. [15] reviewed different methods of obtaining pathogens causing lower respiratory infections. They found the specificity of bronchoscopy to be low due to contamination with upper airway microbiota; however bronchoscopy with BAL improves diagnostic accuracy. Deep coughed sputum is a widely used method of obtaining lower respiratory tract pathogens, yet according to Brook et al. [5], sputum is not reliable for identifying lung pathogens as it is contaminated by bacteria from the oropharynx. Loens et al. reported that sputum cultures can be used but must "be screened by microscopic examination for the relative number of polymorphonuclear cells and squamous epithelium cells" as we did; in addition the results of gram-stained specimens are not always reproducible among different technicians [15]. Due to the retrospective nature of our study over a 25-year period, there might have been a lack of uniformity in methods, although sputum screening for oropharyngeal contaminations was introduced in 1981. This contributes to the limitations of our study. Lastly, Mark et al. [8] reported that organisms obtained via trans-tracheal aspiration, represent oropharyngeal microbiota and not actual lower pulmonary tract pathogens. The pathogens either colonized the trachea prior to the trans-tracheal aspiration or entered during the procedure. It is therefore recommended to obtain lower respiratory tract cultures in ventilated patients via protected specimen brush [8]. Protected specimen brush is a technique in which the brush is placed into a plastic tube before it is inserted or removed from the throat to prevent bacteria in the throat from contaminating the specimen. In our study we used a sterile suction catheter, which was introduced below the vocal cords.

True parenchymal infection by any of the above fungi isolated, is extremely unlikely, and they probably represent colonization, especially following antibiotic therapy.

It is known that the microbiota of patients can change when they are hospitalized or given antibiotics [14]. In a study performed by Wang et al. [16], *S. pneumoniae* was found to be the second most common bacterial pathogen in children with acute pneumonia, however it was rarely found in children who were previously given antibiotics. Additionally, Bartlett et al. [4] found that 67% of patients who acquired their infection during hospitalizations had a mixed culture of both anaerobic and aerobic isolates, compared to 63% of patients who acquired the infection prior to hospitalization and had just anaerobic microbiota. This was mainly seen with Pseudomonas and enteric gram-negative bacilli [4]. Therefore, future studies should take into consideration the antibiotic treatment history.

**Conclusion**

This study demonstrated a model from which we can learn about the pathogens of aspiration to the lung. The results showed that the prevalence of each species differed based on the method of specimen retrieval. The most common bacterial isolate both overall and via BAL-DBL and sputum retrieval was *Haemophilus influenzae*. The most common isolate from ETA was Pseudomonas aeruginosa. Other common organisms included Streptococcus species, coagulase negative Staphylococcus species, and Moraxella catarrhalis. Thus, when treating PD patients with recurrent aspiration with an empiric antibiotic, one should be aware of the bacterial pathogens frequencies according to the method taken. Also, one should take into account the previous sensitivities of the bacteria. Further studies should investigate the sensitivity and specificity of antimicrobial drugs to specific pathogens, prior hospitalizations, severity of lung disease and recurrent antibiotic use.

**Acknowledgement**

We thank the Dysautonomia Foundation Inc., USA for partial support of this study.

**References**


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Received Date: May 21, 2016, Accepted Date: August 02, 2016, Published Date: August 10, 2016.

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