A retrospective review of non-tuberculous mycobacteria in paediatric cystic fibrosis patients at a regional centre

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Non-tuberculous mycobacteria (NTM) are increasingly isolated from CF patients. We carried out a retrospective review of respiratory specimens from paediatric patients at a regional CF centre to assess the possible significance of NTM. Samples from 138 patients were reviewed. 7 patients (4%) were identified as having NTM isolated from at least one clinical specimen (4 male, 3 female, age range when NTM first isolated 7–12 yrs). The sample type of first isolation was a bronchoalveolar lavage (BAL) in 2 cases, sputum in 3 cases and cough swab in 2 cases. The NTM isolated were M. avium complex (MAC) (n = 3), M. chelonae (n = 3) and M. abscessus (n = 1). Initial NTM positive samples were isolated from 1 patient in 2007, 3 patients in 2008, 2 in 2009 and 1 in 2010. None of the initial positive samples (BALs or sputums) were smear positive, although one patient with an initial smear negative, culture positive sputum was smear positive on a follow-up BAL.

All isolates were initially sensitive in vitro to azithromycin and clarithromycin. Only one patient was treated for NTM infection. MAC was isolated from a BAL following deterioration in respiratory symptoms; Burkhodera vietnamiensis was also isolated and he received dual treatment with ciprofloxacin and azithromycin and was showing an improvement in respiratory function at 3 months. All patients had other potential pathogens isolated with NTM, including Aspergillus sp. (n = 4). Three patients received steroids for treatment of ABPA prior to isolation of NTM. Identifying whether NTM represents colonisation or infection is difficult and may be complicated by other organisms; trial of therapy with assessment of response may be indicated.

Reference(s)

Pneumocystis jirovecii, Mycoplasma pneumoniae and Chlamydia pneumoniae carriage rates in adult cystic fibrosis patients in West Midlands, England

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Introduction and Aims: CF and non-CF bronchiectasis are characterised by chronic lung infection and subsequent decline in function. Areas of low oxygen tension have been identified within the lungs of these patients and anaerobes have been isolated with Pneumocystis spp. predominating. Pneumocystis spp. are well characterised oral pathogens, and are also suspected of involvement in pulmonary infection. This study aimed to examine the molecular epidemiology of Pneumocystis spp.

Methods: Isolates were cultured under strict anaerobic conditions from CF sputum (n = 5), oral plaque (n = 5), non-CF sputum (n = 4) and a type strain was acquired from the American Type Culture Collection (ATCC (n = 1)). All isolates were identified by 16s rDNA sequencing and then characterised by PFGE, using XbaI.

Results: Comparison of isolates by PFGE (n = 15) grouped isolates into two pulsortypes [Clusters A (n = 5) and B (n = 8)], with 56% similarity between each cluster. A further 2 isolates had only one band and were not included in the comparison. Each cluster included Pneumocystis from several species (P. melanogensica, P. nigrecens and P. salicis). There appears to be no distinct relationship between disease state and pulsortype. A high degree of inter-species homology, as determined by PFGE (e.g. 91% between P. denticola and P. nigrecens) was apparent. Analysis of isolates (n = 3) in duplicate showed high levels of reproducibility (95–100%).

Conclusion: Initial PFGE studies have identified two main pulsortypes among Pneumocystis spp. examined. Whilst PFGE may provide a useful tool for understanding the molecular epidemiology of clinical Pneumocystis spp. it requires validation using further isolates.

Pneumocystis jirovecii carriage among cystic fibrosis (CF) and non-CF bronchiectasis by pulse field gel electrophoresis (PFGE)

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Objective: To determine whether we could detect the presence and carriage rate of three organisms known to cause atypical infection in immunocompromised hosts, in the sputa of 150 CF patients using sensitive nested PCR.

Methods: 190 sputum samples were collected from 150 patients attending Heartlands Hospital between November 2009–November 2010. Duplicate samples collected were analysed and included in the final analysis. DNA was extracted from these sputa and nested double round PCR for P. jirovecii, M. pneumoniae and C. pneumoniae was performed on all samples, with the results investigated by gel electrophoresis.

Results: 6 patients were found to be PCR positive for P. jirovecii, consistent with a 4.3% carriage rate in this cohort. Repeat samples taken from initially negative patients remained negative on repeat analysis. Induced sputum was obtained from 5 of these patients and re-analysed after 6–8 months. All had become P. jirovecii negative on repeat PCR testing. Furthermore, none of the 190 samples was found to be positive for M. pneumoniae or C. pneumoniae suggesting that CF patients are not carriers of these organisms.

Discussion: We detected a carriage rate of 4.3% for P. jirovecii, this is lower than previously reported (7.4%) [1]. No patients had clinical features of infection with P. jirovecii suggesting that CF patients may act as a transient reservoir for infection. We suggest that P. jirovecii should be considered as a potential pathogen and may warrant treatment if there is poor response to standard therapy.

Characterisation of Prevotella spp. isolated from patients with cystic fibrosis (CF) and non-CF bronchiectasis by pulse field gel electrophoresis (PFGE)

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Objective: To assess the value of bronchoscopy and bronchoalveolar lavage (BAL) in patients with CF and radiological features of ‘atypical infection’.

Method: Clinical, microbiological and radiological data was retrospectively collected for all patients who had undergone bronchoscopy between 2005 and 2010. Data for individuals where the primary indication for BAL was features of ‘atypical infection’ on HRCT was subsequently analysed.

Results: A total of 29 bronchosopies were undertaken. Ten procedures in 8 patients were as a direct result of HRCT appearance. In five, BAL identified a significant pathogen which had not been isolated from sputum.

Table 1

<table>
<thead>
<tr>
<th>Patient</th>
<th>CT Findings</th>
<th>Sputum Findings</th>
<th>BAL Findings</th>
<th>Treatment Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>23/F</td>
<td>CT: Nodules</td>
<td>S. aureus (MRSA)</td>
<td>BAL: MRSA, PA</td>
<td>No Rx change</td>
</tr>
<tr>
<td>30/M</td>
<td>CT: Nodules</td>
<td>S. maltophilia (SM)</td>
<td>BAL: MRSA, PA</td>
<td>No Rx change</td>
</tr>
<tr>
<td>66/M</td>
<td>CT: Nodules</td>
<td>P. aeruginosa (PA)</td>
<td>BAL: PA, AF</td>
<td>AF already on antifungal Rx</td>
</tr>
<tr>
<td>28/F</td>
<td>CT: consolidation, diluted mucous filled bronchi</td>
<td>S. pneumoniae</td>
<td>BAL: No growth on antifungal Rx</td>
<td>No Rx change</td>
</tr>
<tr>
<td>25/M</td>
<td>CT: soft tissue attenuation of endobronchial material</td>
<td>P. melanogenica</td>
<td>BAL: No growth on antifungal Rx</td>
<td>No Rx change</td>
</tr>
<tr>
<td>18/F</td>
<td>CT: Nodules</td>
<td>P. aeruginosa (PA)</td>
<td>BAL: PA, AF</td>
<td>No Rx change</td>
</tr>
<tr>
<td>24/F</td>
<td>CT: Nodules</td>
<td>M. avium (MAC)</td>
<td>BAL: AF</td>
<td>No Rx change</td>
</tr>
<tr>
<td>24/F</td>
<td>CT: Nodules</td>
<td>M. intracellulare</td>
<td>BAL: AF</td>
<td>No Rx change</td>
</tr>
<tr>
<td>24/F</td>
<td>CT: Nodules</td>
<td>P. stutzeri</td>
<td>BAL: AF</td>
<td>No Rx change</td>
</tr>
<tr>
<td>24/F</td>
<td>CT: Nodules</td>
<td>M. terrae</td>
<td>BAL: AF</td>
<td>No Rx change</td>
</tr>
</tbody>
</table>

In six procedures, no ‘atypical organism’ was identified. Only one patient had their treatment changed by BAL, although results were used to confirm diagnosis and continued treatment. The remaining cases had commenced appropriate therapy pre-bronchoscopy guided by sputum microbiology, past medical history and clinical presentation.

Discussion: Treatment changes on HRCT raised the possibility of atypical infection. While the majority of patients had been started on appropriate treatment, BAL was useful in guiding long term management.