Original Article

Bacteria in sputum of patients with chronic chest lesions in chest department of Beni-Suef University Hospital

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KEYWORDS
Chronic chest lesions; Anaerobes; Bacterial infection; Aerobes

Abstract Pulmonary infection in chronic chest lesions (CCL) is poly-microbial and it is possible that anaerobic bacteria that are not detected by routine aerobic culture methods may be present in the airways mucus.

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Introduction

Chronic, pulmonary infections remain the most prominent cause of the increased morbidity and mortality in CCL with more than 95% of deaths due to respiratory failure. The bacteria most frequently isolated from the sputum of patients with CCL and pulmonary infection by standard aerobic microbiological methods are Pseudomonas, Staphylococcus aureus, and Burkholderia cepacia complex.

Recently, it has been shown that there are steep oxygen gradients in the mucus of the patients with CCL leading to proliferation of Pseudomonas within hypoxic media that generates fully hypoxic (anaerobic) conditions in the lungs of patients with CCL with persistent respiratory infection [1]. Because of that there is the real possibility that CCL pulmonary infection is poly-microbial with anaerobic bacteria that are not detected by routine aerobic culture methods.

A number of studies have detected pathogenic anaerobic bacteria in significant numbers in the lungs of patients with CCL by culture [2].

Mainly Pseudomonas and B. cepacia complex are the principle anaerobic pathogens in lungs of patients with CCL such as pneumonia, lung abscesses, and emphysema in which aerobes and anaerobes were present in a poly-microbial infection and were considered to be of significance [3].

The presence of anaerobes in the lungs of patients with CCL could be important because current antibiotic treatment targeting aerobic bacteria may be ineffective against anaerobic bacteria, which may result in a less optimal treatment outcome for patients [3].

In this study, we used strict anaerobic bacteriologic culture techniques to detect anaerobic bacteria in sputum and broncho-alveolar lavage fluid (BAL) to determine the susceptibility of these anaerobes to a range of antibiotics [3].
Aim of the work

- To determine whether anaerobic bacteria are present in the sputum of patients with CCL and its role in pathogenesis of these lesions.
- To detect the antimicrobial susceptibility of anaerobic bacterial infection in CCL patients in Beni-Suef University Hospital.

Patients and methods

There are currently 100 adults attending the chest department in Beni-Suef University Hospital at the time of this study:

- 75 patients had chronic Pseudomonas infection.
- 10 patients had chronic B. cepacia complex infection.
- 15 infected by a number of different pathogens.
- 20 healthy volunteers (control).

All were clinically stable patients who were capable of expectorating sputum sample. Clinical stability was defined as no change in symptoms, FEV1 within 10% of best value in the previous 6 months, and no new antibiotic.

Collection of sputum samples from adult patients with CCL

Sputum samples were collected in sterile containers. After collection, all samples were transferred within 15 min to an anaerobic cabinet in the microbiology laboratory for processing.

Collection of BAL from patients with CCL

BAL samples were frozen within 5–10 min of collection and stored at −70°C. When all samples had been collected, they were transported in dry ice at −70°C to microbiology department for processing. Samples were subsequently thawed in the anaerobic cabinet at 37°C before processing.

Results

<table>
<thead>
<tr>
<th>Aerobic isolates</th>
<th>No of isolates from 100 sputum samples</th>
<th>% of isolates from 100 sputum samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Name</td>
<td>No of isolates</td>
<td>% of isolates</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>B. cepacia complex</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Rothia</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Streptococcus</td>
<td>73</td>
<td>73</td>
</tr>
<tr>
<td>Staphylococcus</td>
<td>31</td>
<td>31</td>
</tr>
<tr>
<td>Microoccus</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>Neisseria</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Bacillus</td>
<td>17</td>
<td>17</td>
</tr>
<tr>
<td>E. coli</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Stenotrophomonas</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Total</td>
<td>282</td>
<td>100</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Anaerobic isolates</th>
<th>Name</th>
<th>No of isolates from 100 sputum samples</th>
<th>% of isolates from 100 sputum samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Name</td>
<td>No of isolates</td>
<td>% of isolates</td>
<td></td>
</tr>
<tr>
<td>Prevotella</td>
<td>29</td>
<td>29</td>
<td></td>
</tr>
<tr>
<td>Actinomyces</td>
<td>10</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Veillonella</td>
<td>7</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Propionibacterium</td>
<td>7</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Peptostreptococcus</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>BuHeidia</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Bifidobacterium</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Gemella</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Lactobacillus</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Fusibacterium</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Clostridium</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Staphylococcus</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Streptococcus</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>62</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>

Discussion

This study provides evidence that the lungs of patients with CCL are not only chronically colonized with pathogens such as Pseudomonas, but also by a range of other bacterial species, many of which are anaerobes. A high percentage of the patients with stable CCL was examined to have numbers of anaerobes in sputum equal to or greater than those of Pseudomonas. In a number of samples, more than one anaerobic species was detected, indicating that the anaerobes exhibited far greater microbial diversity than previously associated with the lung of CCL. Furthermore, we detected similar anaerobic species in multiple samples collected from the same patients at different time points, which suggests persistence of these bacteria within the lung of those patients. These culture results support the results of many molecular studies, which indicated the presence of metabolically active bacterial community within the lung of patients with CCL [4].

The anaerobes detected by culture in the present study and previously by molecular methods were those that would normally be found colonizing the oropharynx [5]. It is possible, therefore, in patients with CCL who have impaired mucociliary clearance mechanisms, that these bacteria are carried from the oropharynx into the lower airways where they colonize the lung and potentially contribute to infection and lung damage. Furthermore, the anaerobes detected were similar to those found in other studies of anaerobic pulmonary infection, such as pneumonia, lung abscesses, and emphysema where aerobes and anaerobes were present in a poly-microbial infection and were considered to be of significance [3].

Colonization with Pseudomonas significantly increased the likelihood that anaerobic bacteria would be present in the sputum. This suggests that a preceding bacterial infection, with Pseudomonas renders airway secretions frankly anaerobic and creates the environment required for subsequent anaerobic infection. Consistent with this hypothesis is the observation...
that proliferation of Pseudomonas within mucus creates anaerobic conditions in the lungs of patients with CCL with persistent respiratory infection. Furthermore, Pseudomonas has been previously shown to rapidly reduce oxygen levels in media during growth under either batch or continuous culture conditions [1].

In addition to aerobic bacteria, such as Pseudomonas and B. cepacia complex, which are assumed to be the key pathogens in CCL pulmonary infection, Streptococcus spp. was also detected in the sputum of patients with CCL. These facultative aerobes would not be routinely considered as primary pathogens in chronic pulmonary infection, with the presence of Streptococcus spp. in the sputum conventionally believed to be as a result of oral contamination [6].

However, in the present study, these bacteria were detected in sputum in numbers equal to Pseudomonas, which suggests that they may be contributing to infection and lung damage. This finding is similar to that reported by DuanK and colleagues [7] who also performed quantitative microbiology on CCL sputum samples and found that the concentration of oropharyngeal strains, such as Streptococcus and Staphylococcus spp., was regularly equal to or higher than that of Pseudomonas. Interestingly, DuanK and colleagues [7] also demonstrated that the presence of Streptococcus spp. isolated from the sputum of patients with CCL enhanced lung damage caused by Pseudomonas infection [7].

Anaerobic bacteria constitute a major component of the normal oral flora; concerns have been expressed regarding contamination of sputum samples by the anaerobic flora during sampling. Indeed, majority of the anaerobic species cultured from sputum in this study have been associated with the oro-pharynx [8].

In this study comparison of the viable count of the anaerobes and Pseudomonas isolated from each sample revealed that, in a high percentage of samples the anaerobe or anaerobes were present in equal or greater numbers than Pseudomonas; because it is recognized as a primary pathogen frequently isolated from sputum, it is extremely unlikely that a significant number of anaerobic bacteria would be acquired during expectoration to equal or exceed the number of Pseudomonas present in the sputum (Table 1).

BAL greatly reduces the risk of oral or upper respiratory tract contamination associated with sputum sample collection [9]. Bronchoscopy was performed using a laryngeal mask airway to further reduce the risk of contamination. Significantly, although present in lower numbers in BAL, we cultured similar anaerobic species in BAL and sputum samples. Given that the total viable count of aerobes detected in BAL was also less in sputum, although only a small number of samples were processed, anaerobes were detected in the lungs of patients in the absence of Pseudomonas. This suggests that anaerobes may cause early infection and then, with time, produce an environment favorable for subsequent infection with Pseudomonas (Table 1).

Control persons’ anaerobes were present in lower numbers and with, different species as compared with CCL and mainly due to contamination from oral flora. These results taken together with the culture results from BAL samples, clearly indicate that the anaerobic bacteria cultured from CCL sputum samples were derived from the lungs and were not contaminants derived from the anaerobic oral flora. This confirms the results of a recent molecular study that compared the bacterial communities in the oral cavity and the lungs of patients with CCL and found that sputum samples were not contaminated to a significant effect by bacterial species found within the oral cavity [4,9].

Although lung function was similar in patients from whom anaerobes were and were not cultured, the presence of anaerobes in the lungs of patients with CCL could still be of important clinical relevance, both as a pure anaerobic infection and in co-infection with Pseudomonas. The anaerobic bacteria that we have detected in the sputum of patients with CCL possess several virulence factors that may be important in the pathogenesis of CCL pulmonary infection. These factors include the following: secretion of a variety of extracellular enzymes, such as proteases and β-lactamase, which may have a detrimental effect on host defense mechanisms and antibiotic response respectively; capsule production biofilm formation neutrophil chemotaxis and resistance to phagocytosis. Furthermore, anaerobes, even when present in low numbers in mixed infections, such as chronic sinusitis and lung abscesses, interact synergistically with aerobic or facultative bacteria and enhance virulence [10].

Moreover, if anaerobic bacteria are contributing to a poly-microbial infection within the lung of CCL, their presence may require a change in the antibiotic CCL used for both treatment of pulmonary exacerbations and for eradication of initial colonization to ensure optimal outcome for the patient [11].

In the present study, the use of chronic maintenance antibiotic therapy had no effect on culture of anaerobic bacteria. This finding was not unexpected given the fact that the antibiotic CCL used primarily for maintenance therapy—clindamycin, azithromycin, and tobramycin—has poor activity against anaerobic bacteria, including those that we have cultured from CCL sputum (Table 2).

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**Table 2**: Antimicrobial susceptibility of anaerobic bacteria isolated from the sputum of adult patients with chronic chest lesions.

<table>
<thead>
<tr>
<th>Name</th>
<th>Ampicillin</th>
<th>Clindamycin</th>
<th>Meropenem</th>
<th>Metronidazole</th>
<th>Piperacillin Tazobactam</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No MIC</td>
<td>MIC</td>
<td>Sus MIC</td>
<td>MIC</td>
<td>MIC</td>
</tr>
<tr>
<td></td>
<td>50% 90% P.</td>
<td>50% 90%</td>
<td>50% 90%</td>
<td>50% 90%</td>
<td>50% 90%</td>
</tr>
<tr>
<td>Prevotella</td>
<td>29 1.05 R</td>
<td>0.19 1.25 R</td>
<td>0.047 0.094 S</td>
<td>0.75 1.62 R</td>
<td>0.0125 0.92</td>
</tr>
<tr>
<td>Veillonella</td>
<td>7 1.55 R</td>
<td>0.125 0.19 S</td>
<td>0.094 0.19 S</td>
<td>0.21 0.32 S</td>
<td>0.38 1.9</td>
</tr>
<tr>
<td>Probionibacterium</td>
<td>7 0.125 S</td>
<td>0.23 0.19 S</td>
<td>0.064 0.25 S</td>
<td>0.19 1.85 R</td>
<td>0.094 0.5</td>
</tr>
<tr>
<td>Actinomycetes</td>
<td>10 1.5 R</td>
<td>0.19 0.16 S</td>
<td>0.032 0.02 S</td>
<td>0.06 0.71 S</td>
<td>0.038 0.06</td>
</tr>
<tr>
<td>Others</td>
<td>9 1.5 R</td>
<td>0.19 1.62 R</td>
<td>0.047 0.125 S</td>
<td>0.71 1.9 R</td>
<td>0.25 0.5</td>
</tr>
</tbody>
</table>
We determined the susceptibility of selected anaerobic isolates to a range of antibiotic with known activity against anaerobes. Because gram-positive anaerobic bacteria, such as Actinomyces and propioni-bacterium species, are commonly resistant to nitroimidazoles, it was not surprising that a high percentage of isolates within these species were resistant to metronidazole. However, the finding that approximately half of the Prevotella isolates examined were resistant to metronidazole was unexpected given that metronidazole resistance among gram-negative anaerobic bacteria is reported to be extremely low. Interestingly, all of the anaerobic isolates tested were susceptible to meropenem, which is used in the treatment of pulmonary exacerbations caused by Pseudomonas, where it shows some superiority over other anti-pseudomonal antibiotic (Table 2).

These findings clearly demonstrate that antibiotic with known activity against anaerobic bacteria may not be effective against anaerobes isolated from CCL sputum and coupled with the need for better patient outcomes and highlight the importance of both culturing anaerobes from sputum and subsequently targeting antibiotic treatment against both anaerobes and aerobes colonizing the lungs of patients with CCL [1].

Conclusion

The results of this study show that a range of potentially pathogenic anaerobic species is present in large numbers of patients with stable CCL. If these anaerobic bacteria are contributing significantly to infection and inflammation in the CCL lung, informed alterations to antibiotic treatment to target anaerobes, in addition to the primary colonizing pathogens, may improve the management of infective exacerbations.

Conflict of interest

No conflict of interest.

References