Evidence of biofilm in chronic suppurative otitis media in Greenland

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Objectives
Chronic suppurative otitis media (CSOM) is very frequent in the Inuit populations all over the Arctic region (1). Risk factors are identified and the microbiology is known (1). Topical antibiotics and irrigation are the treatments of choice and surgery is also possible. Biofilm has recently been claimed to be involved in the pathogenesis of especially chronic middle ear infections and in chronic suppuration after tubulation (ref). Maybe biofilm also play an active role in chronic otitis media with effusion (COME) (2). The nature of CSOM is long lasting episodes of otorhea with varying length of silent periods. Due to this chronic appearance we wanted to investigate CSOM patients in Greenland for presence of biofilm.

Material & Methods
During a 10 days period we examined children with CSOM who met for examination and treatment and children with COME who came for tubulation and adenoidectomy at the yearly ENT-specialist visit in Ammassalik district in East Greenland.

- 6 children with CSOM
- 4 children with COME
- Sterile aspiration of middle ear pus and effusions
- Smears were immediately prepared on slides and heat fixated
- Culture swabs was stored in Stuarts transportmedium until culturing at a Microbiological Lab. in Denmark
- Gram-staining and conventional blinded microscopy
- PNA-FISH = Peptide Nucleic Acid Fluorescens In Situ

Hybridization specific for S. aureus and E. coli was applied

Results
The CSOM children were 9 - 15 years old (5 girls and 1 boy). The COME children were 2 - 5 years old (2 girls and 2 boys). We found microscopic evidence of biofilm formation in all six CSOM specimens but in none of the seven COME specimens (bilateral from three patients). In four CSOM children we cultured S. aureus with corresponding Gram-pos. cocci in the biofilms and in two we cultured E. coli with Gram-negative rods in the biofilms. All COME patients were culture negative.

<table>
<thead>
<tr>
<th>Age (yrs)</th>
<th>Diag.</th>
<th>Culture</th>
<th>Biofilm</th>
<th>PNA-FISH</th>
<th>biofilm</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>CSOM dx</td>
<td>S. aureus</td>
<td>Yes</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>CSOM sin</td>
<td>S. aureus</td>
<td>Yes</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>CSOM dx</td>
<td>E. coli</td>
<td>Yes</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>CSOM sin</td>
<td>E. coli</td>
<td>Yes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>CSOM dx</td>
<td>S. aureus</td>
<td>Yes</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>2,3,3,5</td>
<td>COME</td>
<td>none</td>
<td>No</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Examples of biofilm in CSOM aspirates

Examples of biofilm in CSOM aspirates

<table>
<thead>
<tr>
<th>Gram-stained specimen</th>
<th>PNA-FISH specimen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (no garlic)</td>
<td>+ tobramycin 24h</td>
</tr>
<tr>
<td>Medium 1% garlic extract (blocks biofilm)</td>
<td>+ tobramycin 24h</td>
</tr>
</tbody>
</table>

Future perspectives
- Surgical eradication - tubulation
- Quorum sensing blockers (e.g. garlic) or read marine-alges (Furanones)
- Polysaccharid matrix degraders – algianases
- Biofilm bacteria release blockers
- Probiotics like lactobacillus in UVI
- Bacteriophag treatment

Conclusions
The findings support the clinical appearance and the difficulties in the treatment of CSOM. Future anti-biofilm antibiotics may be of value in the treatment of CSOM.

- Biofilm is involved in the pathogenesis of CSOM
- Biofilm can explain the clinical course of chronic ear infections
- The treatment of chronic ear infections will most likely change in the future according to the biofilm biology
- More investigations are wanted

References