Microbial interactions in an endotracheal biofilm

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• Why study the ETT biofilm?
• The ETT microbiome: molecular analysis and impact on development of ventilator-associated pneumonia (VAP)
• Mono- and -polymicrobial VAP
• Modelling ETT interactions
• Conclusions
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Why study the ETT biofilm?
Biofilm formation on the endotracheal tube

Intubated patient: A: endotracheal tube; B: cuff inflation tube with pilot balloon; C: trachea; D: esophagus; E: cuff; Patient material and micro-organisms can easily accumulate in the lower part of the ET tube around the cuff.

Bacteria discarded from the biofilm can make it to the lung and cause ventilator associated pneumonia (VAP).

P. aeruginosa
S. marcescens
Other bacteria
Bacterial colonization of the ETT


Colonisation of the upper respiratory tract with Gram-negative bacilli after operation, endotracheal intubation and prophylactic antibiotic therapy.

Redman LR, Lockey E.


Nosocomial pulmonary infection: possible etiologic significance of bacterial adhesion to endotracheal tubes.

Sottile FD, Marrie TJ, Prough DS, Hobgood CD, Gower DJ, Webb LX, Costerton JW, Griswold AG.

Abstract
Biomaterials are essential for life support and monitoring of critically ill patients, but their use increases the risk of nosocomial infection. Of the various plastics used for life support and monitoring devices, polyvinylchloride is one to which bacteria most readily adhere. Through the use of qualitative culture techniques and scanning and transmission electron microscopy, we studied the surfaces of polyvinylchloride endotracheal tubes removed from 25 ICU patients, to determine if bacterial adhesion to those tubes was sufficient to provide a possible source for repeated contamination of the tracheobronchial tree. Of the surfaces studied, 16% were partially covered and 84% were completely covered by an amorphous bacteria-containing matrix. Some biofilm-enclosed bacterial aggregates projected from the matrix into the lumen of the tube. The mechanism by which endotracheal tubes repeatedly inoculate the lungs of intubated patients may prove to be dislodgment of such aggregates by suction apparatus.
Is it really a biofilm?

14 hrs post-intubation

3 days post-intubation

Sottile et al, 1986; Zur et al, 2004
Table 2 Numbers of isolates of potential pathogens recovered from endotracheal tube biofilm and tracheal secretions

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Control</th>
<th>VAP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Biofilm</td>
<td>Tracheal</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>EGNB</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td><em>Enterococcus faecalis</em></td>
<td>2</td>
<td>–</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>2</td>
<td>–</td>
</tr>
<tr>
<td><em>Candida spp.</em></td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>
ETT biofilms are associated with microbial persistence and treatment failures

Table 2 Bacterial isolation in surveillance endotracheal aspirates

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>ETA n, %</th>
<th>Days until ETA+ (mean ± SEM)</th>
<th>ETA-ETT match (n, %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colonized patients</td>
<td>65, 87%</td>
<td>2.1 ± 0.4</td>
<td>36, 56%</td>
</tr>
<tr>
<td>Acinetobacter baumannii</td>
<td>20, 32%</td>
<td>7.8 ± 1.6</td>
<td>12, 60%</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>14, 22%</td>
<td>5.4 ± 2.1</td>
<td>9, 64%</td>
</tr>
<tr>
<td>Cocci (SCN, Streptococcus spp)</td>
<td>13, 20%</td>
<td>5.0 ± 0.9</td>
<td>4, 31%</td>
</tr>
<tr>
<td>Staphylococcus aureus (MSSA,MRSA)</td>
<td>10, 15%</td>
<td>2.2 ± 0.6</td>
<td>6, 60%</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>29, 45%</td>
<td>2.0 ± 0.6</td>
<td>6, 21%</td>
</tr>
<tr>
<td>Candida no albicans</td>
<td>17, 26%</td>
<td>3.2 ± 0.5</td>
<td>1, 6%</td>
</tr>
</tbody>
</table>

Gil-Perotin et al, Crit. Care, 2012
• Why study the ETT biofilm?
• **The ETT microbiome: molecular analysis and impact on development of ventilator-associated pneumonia (VAP)**
• Mono- and -polymicrobial VAP
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Molecular analysis of microbial communities in ETT biofilms

- 20 ETTs were analysed by 16S rDNA PCR-cloning-Sanger sequencing and 4 by pyrosequencing
- On average, 16S rDNA sequencing revealed 3 different species per ET biofilm
  - Simpson diversity indexes did not differ significantly between both methods (culture and sequencing of the clone libraries)
- Pyrosequencing analysis suggested that the four samples were dominated by members of the normal oral flora such as *Prevotella* spp., *Peptostreptococcus* spp. and lactic acid bacteria
Molecular analysis of microbial communities in ETT biofilms

- Inner luminal surface of 24 ETT from 20 patients analysed by quantitative culture and by denaturing gradient gel electrophoresis (DGGE) profiling of 16S rRNA gene

- DGGE profiling of the endotracheal biofilms revealed complex banding patterns containing between 3 and 22 (mean 6) bands per tube

- Significant inter-patient diversity

- No. of DGGE bands detected was not related to total viable microbial counts or the duration of intubation
Prevalence of potential pathogens in ET tube biofilms

Hotterbeekx et al, In preparation, 2015
Analysis of ETTs culture positive for *P. aeruginosa* or *S. epidermidis* or both

Sample processing workflow:
- Collection of ET
  - Mechanically ventilated patients in ICU
- Culture of ET
  - Blood agar, 37°C
  - Identification by MALDI-TOF
  - Selection of 39 ET positive for *P. aeruginosa* and/or *S. epidermidis*
- 16S sequencing
  - DNA extraction: Masterpure complete DNA and RNA purification kit (Epicentre)
  - Target: V3-V5 region
  - Platform: Roche 454

Pre-processing:
- MG-RAST online software (default parameters)
- Template alignment and quality control
- Chimera removal

Sequence analysis:
- BLAT similarity search against M5ma database
- Min% identity cutoff 97%
- Min alignment cutoff 15

Microbiome discovery:
- Common core based on presence/absence of family (50% cutoff)
- Differences in relative abundance between groups based on LefSe2 and LDA2

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Hotterbeekx et al, In preparation, 2015
Histology of ETT biofilms

- Microtome sectioning
- Paraffin embedding
- Staining
  - H&E
  - Gram
  - DAPI

Courtesy S. Kumar-Singh, Vaxinfectio, Univ. Antwerp
Histology of ETT biofilms

Courtesy S. Kumar-Singh, Vaxinjectio, Univ. Antwerp
<table>
<thead>
<tr>
<th>Age (years)</th>
<th>APACHE II</th>
<th>Ventilation days</th>
<th>BAL culture</th>
<th>ETT culture</th>
</tr>
</thead>
<tbody>
<tr>
<td>55</td>
<td>35</td>
<td>15</td>
<td>Pa</td>
<td>Pa</td>
</tr>
<tr>
<td>55</td>
<td>7</td>
<td>23</td>
<td>Pa</td>
<td>Pa</td>
</tr>
<tr>
<td>58</td>
<td>24</td>
<td>12</td>
<td>Pa</td>
<td>Pa</td>
</tr>
<tr>
<td>42</td>
<td>21</td>
<td>6</td>
<td>Staph spp.</td>
<td>C. glabrata</td>
</tr>
<tr>
<td>68</td>
<td>31</td>
<td>22</td>
<td>Staph spp.</td>
<td>C. albicans</td>
</tr>
<tr>
<td>74</td>
<td>30</td>
<td>8</td>
<td>C. aureus</td>
<td>K. pneumonia</td>
</tr>
<tr>
<td>58</td>
<td>14</td>
<td>7</td>
<td>C. aureus</td>
<td>K. pneumonia</td>
</tr>
<tr>
<td>31</td>
<td>22</td>
<td>19</td>
<td>Staph spp.</td>
<td>C. glabrata</td>
</tr>
<tr>
<td>47</td>
<td>22</td>
<td>8</td>
<td>C. aureus</td>
<td>C. freundii</td>
</tr>
<tr>
<td>49</td>
<td>23</td>
<td>23</td>
<td>K. pneumonia</td>
<td>C. albicans</td>
</tr>
<tr>
<td>39</td>
<td>20</td>
<td>19</td>
<td>E. coli</td>
<td>L. paracasei</td>
</tr>
<tr>
<td>59</td>
<td>17</td>
<td>16</td>
<td>Pa</td>
<td>Pa</td>
</tr>
<tr>
<td>58</td>
<td>35</td>
<td>5</td>
<td>E. coli</td>
<td>Pa</td>
</tr>
</tbody>
</table>

Patients who developed VAP:

- Patient 1
- Patient 2
- Patient 3
- Patient 4
- Patient 5
- Patient 6
- Patient 7
- Patient 8
- Patient 9
- Patient 10
- Patient 11
- Patient 12
- Patient 13
- Patient 14
- Patient 15

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Characterizing the ETT biofilm microbiome

S. epidermidis: 290 spp in total and 35 spp/tube on average

P. aeruginosa: 116 spp in total and 20 spp./tube

P. ae + S. epi: 111 spp. In total and 29 spp./tube

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Core ETT microbiome

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Organisms associated with *S. epidermidis* in ETT biofilms

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Development of VAP and patient survival upon extubation: a cluster analysis

Cluster 1 (n=16)
- Patient survival: 100% survived
- VAP status: 81.2% VAP
- Biomass visible on ETT: 50%
- Culture results: 56.2% S. epidermidis
- APACHE II: 25.50
- Age: 57.69
- Duration of intubation: 13.69 days

Cluster 2 (n=11)
- Patient survival: 100% died
- VAP status: 81.8% non-VAP
- Biomass visible on ETT: 81.8%
- Culture results: 63.6% P. aeruginosa
- APACHE II: 26.55
- Age: 67.82
- Duration of intubation: 12.45 days

Cluster 3 (n=12)
- Patient survival: 100% survived
- VAP status: 100% non-VAP
- Biomass visible on ETT: 100%
- Culture results: 66.7% S. epidermidis
- APACHE II: 18.50
- Age: 60.50
- Duration of intubation: 11.42 days

Can the bacterial consortium on the ETT influence or help predict patient outcome?

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Impact of the ETT microbiome on patient survival

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Is ICU pneumonia (VAP) polymicrobial?

- 3 year study in HAP and CAP patients and controls
- 25% BAL samples monomicrobial and 75% polymicrobial from pneumonia patients

Bousbia et al, PLOS One, 2012
Characteristics of mono- and polymicrobial VAP

<table>
<thead>
<tr>
<th></th>
<th>Pneumonia patients (n = 135/185)</th>
<th>Control subjects (n = 22/25)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Monomicrobial</td>
<td>Polymicrobial</td>
</tr>
<tr>
<td>Case number</td>
<td>32</td>
<td>82</td>
</tr>
<tr>
<td>Temperature, °C (SD)</td>
<td>37.6 (1)</td>
<td>37.8 (1.7)</td>
</tr>
<tr>
<td>Initial antibiotic therapy</td>
<td><strong>18</strong></td>
<td><strong>33</strong></td>
</tr>
<tr>
<td>Less than 2 days prior to sampling</td>
<td>6</td>
<td>17</td>
</tr>
<tr>
<td>3 days or more prior to sampling</td>
<td>12</td>
<td>16</td>
</tr>
<tr>
<td>Length of ICU stay prior to sampling, d (SD)</td>
<td><strong>6.1 (9.1)</strong></td>
<td><strong>6.3 (10)</strong></td>
</tr>
<tr>
<td>Total length of hospital stay, d (SD)</td>
<td><strong>26 (29.7)</strong></td>
<td><strong>28.2 (23.3)</strong></td>
</tr>
<tr>
<td>Length of MV prior to sampling, d (SD)</td>
<td><strong>6.8 (10.3)</strong></td>
<td><strong>7.1 (11.2)</strong></td>
</tr>
<tr>
<td>Sepsis</td>
<td>7</td>
<td>19</td>
</tr>
<tr>
<td>Septic shock</td>
<td>15</td>
<td>38</td>
</tr>
<tr>
<td>ICU mortality (%)</td>
<td><strong>16 (50)</strong></td>
<td><strong>23 (28)</strong></td>
</tr>
</tbody>
</table>

Pie charts show the distribution of bacterial groups:

- CAP: Bacilli (13%), Bacteriodia (13%), Gamma proteobacteria (13%), etc.
- VAP: Bacilli (20%), Bacteriodia (18%), Gamma proteobacteria (14%), etc.
- CS: Bacteriodia (21%), Bacilli (18%), Clostridia (13%), Gamma proteobacteria (16%), etc.

Bousbia et al, PLOS One, 2012
Is ICU pneumonia (VAP) polymicrobial?

Bousbia et al, PLOS One, 2012

*P. aeruginosa* common to both pneumonia patients and controls: core pulmonary microbiota?
Microbial consortium interactions: ‘Keystone’ and dominant pathogens

Why study the ETT biofilm?

The ETT microbiome: molecular analysis and impact on development of ventilator-associated pneumonia (VAP)

Mono- and polymicrobial VAP

Modelling ETT interactions

Conclusions
In vitro modelling of ETT microbial interactions

De Backer et al, 2015
In vitro modelling of ETT microbial interactions

- S. epidermidis / C. albicans: synergism
- S. epidermidis / S. marcescens
- S. epidermidis / K. pneumoniae
- S. epidermidis / P. aeruginosa: antagonism
Effect of biofilm supernatant of *P. aeruginosa* and *S. marcescens* on *S. epidermidis* biofilms

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In vivo modelling of ETT microbial interactions

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Courtesy S. Kumar-Singh, Vaxinfectio, Univ. Antwerp
Some conclusions

- Need for more comprehensive ‘Big data’
  - Role of accessory organisms in VAP etiology
- ETT fingerprint as a prognostic and, possibly, diagnostic marker
- Rapid molecular diagnostic tools to include fastidious ‘keystone’ and ‘dominant’ pathogens