In vitro pharmacodynamic models for the study of antibiotic activity against bacterial biofilms

Françoise Van Bambeke, PharmD, PhD

Pharmacologie cellulaire et moléculaire
Louvain Drug Research Institute

Université catholique de Louvain, Brussels, Belgium
Biofilms in human infections

Biofilms are associated to $65^a$-$80^b$ % of human infections and can colonize virtually all organs ...

\[\text{ear, nose, throat, mouth & teeth, eye, lung, heart, kidney, gall bladder, pancreas, nervous system, skin, bone, \text{*** implanted medical devices}}\]

\(^a\text{CDC 1999; }^b\text{Lewis et al, Nat Rev Microbiol. 2007; 5:48-56}\]
Antibiotics and biofilms in clinical practice

Reduced Vancomycin Susceptibility in an In Vitro Catheter-Related Biofilm Model Correlates with Poor Therapeutic Outcomes in Experimental Endocarditis Due to Methicillin-Resistant Staphylococcus aureus

The presence of antibiotic-resistant nosocomial pathogens in endotracheal tube biofilms and corresponding surveillance cultures.

Treatment failure is not rare...
PK/PD parameters in biofilms

- **pharmacokinetics**
  - diffusibility through the matrix
  - access to bacteria
  - efflux out of bacteria

- **pharmacodynamics**
  - bacterial responsiveness (metabolic activity of bacteria)
  - antibiotic expression of activity (local environment [O₂, pH, ..])

**nutrients & oxygen**

catheter, bone, skin, cardiac valve, ..
Main pathogens in biofilm-related diseases

<table>
<thead>
<tr>
<th>Bacterial species</th>
<th>Biofilm infection</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Escherichia coli</strong></td>
<td>Acute and recurrent urinary tract infection, catheter-associated urinary tract infection, biliary tract infection</td>
</tr>
<tr>
<td><strong>Pseudomonas aeruginosa</strong></td>
<td>Cystic fibrosis lung infection, chronic wound infection, catheter-associated urinary tract infection, chronic rhinosinusitis, chronic otitis media, contact lens-related keratitis</td>
</tr>
<tr>
<td><strong>Staphylococcus aureus</strong></td>
<td>Chronic osteomyelitis, chronic rhinosinusitis, endocarditis, chronic otitis media, orthopaedic implants</td>
</tr>
<tr>
<td><strong>Staphylococcus epidermidis</strong></td>
<td>Central venous catheter, orthopaedic implants, chronic osteomyelitis</td>
</tr>
<tr>
<td><strong>Streptococcus pneumoniae</strong></td>
<td>Colonization of nasopharynx, chronic rhinosinusitis, chronic otitis media, chronic obstructive pulmonary disease</td>
</tr>
<tr>
<td><strong>Streptococcus pyogenes</strong></td>
<td>Colonization of oral cavity and nasopharynx, recurrent tonsilitis</td>
</tr>
</tbody>
</table>
Quantifying biomass and metabolic activity in biofilms

**biofilm mass**

**crystal violet**

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**Gram(+) bacteria**

**metabolic activity**

**Gram(-) bacteria**

**resazurin**

**fluorescein diacetate**

**resorufin**

**fluorescein**
S. aureus & S. pneumoniae models

Kinetics of biofilm formation

**S. aureus**

- Crystal violet absorbance
- Resorufin fluorescence

**S. pneumoniae**

- ATCC 49619
- R6

Young biofilm | Mature biofilm
---|---
Young biofilm | Mature biofilm
Pharmacodynamic model for antibiotic activity

An example with young biofilm of *S. aureus*

*Bauer, Siala et al, Antimicrob Ag Chemother. 2013;57:2726-37*
S. aureus (MRSA) mature biofilms

more effective and more potent on viability than on matrix

RIF and DAP more efficient at clinically achievable concentrations

*Bauer, Siala et al, Antimicrob Ag Chemother. 2013;57:2726-37*
S. aureus (MRSA) mature biofilms

Moxifloxacin and *S. pneumoniae* biofilms


maximal efficacy \(\uparrow\) with maturity
Moxifloxacin and *S. pneumoniae* biofilms


relative potency \(\updownarrow\) with maturity
Comparison of PD parameters

**S. aureus isolates from persistent infections**

**Reference strain (ATCC33591)**

**Clinical isolate (2005/104)**
Antibiotic potency against clinical isolates of *S. aureus*

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**daptomycin**

**moxifloxacin**

**vancomycin**

<table>
<thead>
<tr>
<th>Concentration (µg/mL)</th>
<th>REDUCTION IN VIABILITY</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>25%</td>
</tr>
<tr>
<td>10</td>
<td>50%</td>
</tr>
<tr>
<td>1</td>
<td>75%</td>
</tr>
</tbody>
</table>

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Legend:
- 1083
- S025
- S027
- 651
- 104
- 179
- S028
Antibiotic potency against clinical isolates of *S. aureus*

**Bodipy-DAP CTC**

**Daptomycin**

**Moxifloxacin**

**Vancomycin**

**Bodipy-DAP CTC**
Antibiotic potency against clinical isolates of *S. aureus*

**Bodipy-VAN CTC**

**ATCC25923 vs vancomycin-bodipy**

**104 vs vancomycin-bodipy**

**vancomycin**

- **1083**
- **S025**
- **S027**
- **651**
- **104**
- **179**
- **S028**

**REDUCTION IN VIABILITY**

- **25%**
- **50%**
- **75%**

**VAN fluorescence**

- **Biofilm depth (µm)**

**20/12/2013**

BIBR - LLN
Anti-biofilm strategies ....

Reversible-irreversible attachment
- Antiadhesion agents (e.g., mannosides, pilicides, and curlicides in inhibition of UPEC biofilms)
- Antibiofilm polysaccharides
- Signal transduction interference

Microcolony formation
- Lytic phages
- Silver nanoparticles
- EPS-degrading enzymes
- Antimicrobial peptides
- Antibiofilm polysaccharides
- Signal transduction interference
- DNAse I, Dispersin B
- Chelating agents

Biofilm maturation
- Lytic phages
- Silver nanoparticles
- EPS-degrading enzymes
- Antimicrobial peptides
- Antibiofilm polysaccharides
- Signal transduction interference
- DNAse I, Dispersin B
- Chelating agents

Dispersal
- c-di-GMP engineering to promote motility versus sessility
- Introduction of dispersing signals (e.g., d-amino acids/norspermidine in the case of B. subtilis)

PK-related parameters: improving diffusion

![Graphs showing the reduction in viability for daptomycin and daptomycin & norspermine concentrations.](image)
PK-related parameters: improving diffusion

651 Daptomycin-bodipy

DAP fluorescence

Biofilm depth (µm)

Daptomycin & norspermine

Bodipy-DAP

CTC
PK-related parameters: improving diffusion

- **Daptomycin-bodipy**
  - Fluorescence vs Biofilm depth (µm)
  - Reduction in Viability

- **Daptomycin & norspermine**
  - Fluorescence vs Biofilm depth (µm)
  - Reduction in Viability

- **S028 vs daptomycin-bodipy**

- **Bodipy-DAP**
  - CTC
PK-related parameters: improving diffusion
PD-related parameters: pH effect?

ATCC33591 (MRSA)

moxifloxacin

Siala et al, Eurobiofilms 2013
PD-related parameters: pH effect?

ATCC33591 (MRSA)

moxifloxacin

biofilm depth (µm)

pH

2005/104

REDUCTION IN VIABILITY

25% 50% 75%

25%

50%

75%

basic

acidic

Siala et al, Eurobiofilms 2013
PD-related parameters: pH effect?

**ATCC33591 (MRSA)**

**2005/104**

**2005/179**

**Moxifloxacin**

- 1083
- △ S025
- ▼ S027
- ◆ 651
- ● 104
- □ 179
- △ S028

**Reduction in Viability**

- Basic
- Acidic

*Siala et al, Eurobiofilms 2013*
PD-related parameters: pH effect?

ATCC33591 (MRSA)

2003/651

2005/104

2005/179

Moxifloxacin

Reduction in viability

Siala et al, Eurobiofilms 2013
**P. aeruginosa biofilms**

Kinetics of biofilm formation

beef infusion solids, casein hydrolysate, starch

mucin, DNA, DTPA, casaminoacids, NaCl, KCl, tris base, egg yolk emulsion.

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**Kinetics of biofilm formation**

- **MHB**
  - Beef infusion solids, casein hydrolysate, starch

- **ASM**
  - Mucin, DNA, DTPA, casaminoacids, NaCl, KCl, tris base, egg yolk emulsion
Antibiotics and *P. aeruginosa* mature biofilms

classes currently used by inhalation in cystic fibrosis patients

aminoglycosides >> polymyxins
Antibiotics and *P. aeruginosa* mature biofilms

Classes in development for inhalation in cystic fibrosis patients

Fluoroquinolones >> β-lactams
Macrolides and biofilms

Aminoglycosides in combination with macrolides

Basseres et al, Pseudomonas meeting 2013
Conclusions and perspectives

- Antibiotic efficacy and relative potency globally reduced against biofilms

- Effect on viability >> effect against biomass in G(+) affecting PK (drug access) and PD (bacterial responsiveness)

- MICs do not predict activity → appropriate models required for screening

- Strain-specific factors affecting PK (drug access) and PD (bacterial responsiveness)

- Combination of antibiotics with agents acting on QS or matrix may be useful

- Mature biofilms much more resistant to antibiotics
Thank you for your attention and ....

Merry Christmas!