Antibiotics and biofilm: in vitro evidence and new clinical applications

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Biofilms: what are we speaking about?

Attachment → Maturation → Dispersion
History of biofilm development *in vivo*

Natural history of biofilm formation *in vivo* during the establishment of chronic implant-associated *S. aureus* osteomyelitis in mice

<table>
<thead>
<tr>
<th>SH1000</th>
<th>15,000X</th>
<th>5,000X</th>
<th>Before insertion</th>
<th>1 day</th>
<th>3 days</th>
<th>7 days</th>
<th>14 days</th>
<th>28 days</th>
</tr>
</thead>
</table>

Attachment → Maturation → Dispersion

*Nishitani et al; J Orthop Res. 2015;33:1311-9*
Biofilms in human infections

Biofilms are associated to 65\textsuperscript{a}-80\textsuperscript{b} \% of human infections and can colonize virtually all organs …

\textbf{Sites of Primary and Secondary Biofilm Infection}

- ear
- nose
- throat
- mouth & teeth
- eye
- lung
- heart
- kidney
- gall bladder
- pancreas
- nervous system
- skin
- bone
- implanted medical devices

\textsuperscript{a}CDC 1999; \textsuperscript{b}Lewis et al, Nat Rev Microbiol. 2007; 5:48-56
## Main pathogens in biofilm-related diseases

<table>
<thead>
<tr>
<th>Bacterial species</th>
<th>Biofilm infection</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em></td>
<td>Acute and recurrent urinary tract infection, catheter-associated urinary tract infection, biliary tract infection</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></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><em>Staphylococcus epidermidis</em></td>
<td>Central venous catheter, orthopaedic implants, chronic osteomyelitis</td>
</tr>
<tr>
<td><em>Streptococcus pneumoniae</em></td>
<td>Colonization of nasopharynx, chronic rhinosinusitis, chronic otitis media, chronic obstructive pulmonary disease</td>
</tr>
<tr>
<td><em>Streptococcus pyogenes</em></td>
<td>Colonization of oral cavity and nasopharynx, recurrent tonsilitis</td>
</tr>
</tbody>
</table>
Antibiotics and biofilms in clinical practice

When and how should we treat biofilms in chronic sinusitis?
Jain R, Douglas R.

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
Wessam Abdelhady, Arnold S. Bayer, Kari Seldl, Cynthia C. Nast, Megan R. Kiedrowski, Alexander R. Harwitt, Michael R. Yeaman, Yan Q. Xiong

Biofilm formation or internalization into epithelial cells enable Streptococcus pyogenes to evade antibiotic eradication in patients with pharyngitis
Takagi Ogawa, Yutaka Terai, Hisashi Okuni, Keiko Ninomiya, Hiroshi Sakata

The presence of antibiotic-resistant nosocomial pathogens in endotracheal tube biofilms and corresponding surveillance cultures.
Vandecandelaere I, Matthijs N, Nelis HJ, Depuydt P, Coene T.

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

**Pharmacokinetics**
- diffusibility through the matrix
- bioavailability within the biofilm
- 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,..

Janssen, Nature 2009
In vitro evidence: models in 96-well polystyrene plates

appropriate dyes
to evaluate biomass or bacterial load
Quantifying biomass and metabolic activity in biofilms

Crystal violet

Biofilm mass

Quantifying biomass and metabolic activity in biofilms

Biofilm mass

Crystal violet

Gram(+) bacteria

Resazurin

Metabolic activity

Gram(-) bacteria

Resorufin

Fluorescein diacetate


Kinetics of biofilm formation

Pharmacodynamic model for antibiotic activity

An example with a young biofilm of *S. aureus* - ATCC MSSA

*Bauer, Siala et al, Antimicrob Ag Chemother. 2013;57:2726-37*
Pharmacodynamic model for antibiotic activity

Young vs. mature biofilm of *S. aureus* - ATCC MSSA

vancomycin vs. young biofilm (6h)

vancomycin vs mature biofilm (24h)

*Bauer, Siala et al, Antimicrob Ag Chemother. 2013;57:2726-37*
Pharmacodynamic model for antibiotic activity

Comparison of antibiotic efficacy – ATCC reference strains

Daptomycin, a lipoglycopeptide

Structure and modes of action of the lipopeptide daptomycin

Daptomycin (lipopeptide)

Van Bambeke et al; Armstrong & Cohen – Infectious diseases 2016
Pharmacodynamic model for antibiotic activity

Comparison of antibiotic relative potency - ATCC MSSA

Delafloxacin, a new fluoroquinolone

DRUG EVALUATION

Delafloxacin, a non-zwitterionic fluoroquinolone in Phase III of clinical development: evaluation of its pharmacology, pharmacokinetics, pharmacodynamics and clinical efficacy

Françoise Van Bambeke*

ABSTRACT Delafloxacin is a fluoroquinolone lacking a basic substituent in position 7. It shows MICs remarkably low against Gram-positive organisms and anaerobes and similar to those of ciprofloxacin against Gram-negative bacteria. It remains active against most fluoroquinolone-resistant strains, except enterococci. Its potency is further increased in acidic environments (found in many infection sites). Delafloxacin is active on staphylococci growing intracellularly or in biofilms. It is currently evaluated as an intravenous and intravenous/oral stepdown therapy in Phase III trials for the treatment of complicated skin/skin structure infections. It was also granted as Qualified Infectious Disease Product for the treatment of acute bacterial skin and skin structure infections and community-acquired bacterial pneumonia, due to its high activity on pneumococci and atypical pathogens.
Delafloxacin, a new fluoroquinolone

DRUG EVALUATION

Delafloxacin, a non-zwitterionic fluoroquinolone in Phase III of clinical development: evaluation of its pharmacology, pharmacokinetics, pharmacodynamics and clinical efficacy

Table 1. Susceptibility of relevant Gram-positive pathogens to delafloxacin and other commercially available fluoroquinolones.

<table>
<thead>
<tr>
<th>Species</th>
<th>Phenotype</th>
<th>Number of strains</th>
<th>Antibiotic</th>
<th>MIC&lt;sub&gt;50&lt;/sub&gt; (mg/l)</th>
<th>MIC&lt;sub&gt;90&lt;/sub&gt; (mg/l)</th>
<th>MIC range (mg/l)</th>
<th>Ref.†</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. aureus</td>
<td>All</td>
<td>681</td>
<td>Levofloxacin</td>
<td>0.12</td>
<td>&gt;32</td>
<td>0.03→32</td>
<td>[41]</td>
</tr>
<tr>
<td></td>
<td>681</td>
<td></td>
<td>Delafloxacin</td>
<td>0.12</td>
<td>0.5</td>
<td>≤0.004→16</td>
<td>[41]</td>
</tr>
<tr>
<td></td>
<td>FQ-S</td>
<td>70</td>
<td>Levofloxacin</td>
<td>0.25</td>
<td>0.5</td>
<td>0.06→0.5</td>
<td>[23]</td>
</tr>
<tr>
<td></td>
<td>88</td>
<td></td>
<td>Moxifloxacin</td>
<td>0.06</td>
<td>0.1</td>
<td>0.015→0.5</td>
<td>[23]</td>
</tr>
<tr>
<td></td>
<td>70</td>
<td></td>
<td>Delafloxacin</td>
<td>0.004</td>
<td>0.008</td>
<td>0.002→0.008</td>
<td>[23]</td>
</tr>
<tr>
<td></td>
<td>88</td>
<td></td>
<td>FQ-R</td>
<td>0.002</td>
<td>0.008</td>
<td>≤0.001→0.06</td>
<td>[42]</td>
</tr>
<tr>
<td></td>
<td>71</td>
<td></td>
<td>Levofloxacin</td>
<td>16</td>
<td>32</td>
<td>4→64</td>
<td>[23]</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td></td>
<td>Moxifloxacin</td>
<td>4.00</td>
<td>8.00</td>
<td>2→32</td>
<td>[23]</td>
</tr>
<tr>
<td></td>
<td>70</td>
<td></td>
<td>Delafloxacin</td>
<td>0.25</td>
<td>1.00</td>
<td>0.015→1</td>
<td>[23]</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td></td>
<td>FQ-R</td>
<td>0.006</td>
<td>0.12</td>
<td>0.015→2</td>
<td>[42]</td>
</tr>
</tbody>
</table>
Comparison of antibiotic activity in confocal microscopy

Live/dead staining (antibiotics at 32 X MIC) – ATCC MRSA

# Moving to clinical isolates from pandemic lineages

### Description of clinical strains included in the study

<table>
<thead>
<tr>
<th>Strain</th>
<th>16S&lt;sup&gt;a&lt;/sup&gt;</th>
<th>nuc&lt;sup&gt;a&lt;/sup&gt;</th>
<th>meca&lt;sup&gt;b&lt;/sup&gt;</th>
<th>spa type&lt;sup&gt;c&lt;/sup&gt;</th>
<th>MLST&lt;sup&gt;d&lt;/sup&gt;</th>
<th>TSST-1&lt;sup&gt;e&lt;/sup&gt;</th>
<th>PVL&lt;sup&gt;e&lt;/sup&gt;</th>
<th>Clinical origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>2011S027</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>t002</td>
<td>CC5</td>
<td>+</td>
<td>−</td>
<td>Cellulitis and bacteremia</td>
</tr>
<tr>
<td>Surv 2003/1083</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>t002</td>
<td>CC5</td>
<td>+</td>
<td>−</td>
<td>Chirurgical wound</td>
</tr>
<tr>
<td>Surv 2005/104</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>t002</td>
<td>CC5</td>
<td>−</td>
<td>−</td>
<td>Skin</td>
</tr>
<tr>
<td>2009S028</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>t002</td>
<td>CC5</td>
<td>+</td>
<td>−</td>
<td>Nasal carriage</td>
</tr>
<tr>
<td>2009S025</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>t002</td>
<td>CC5</td>
<td>+</td>
<td>−</td>
<td>Ear</td>
</tr>
<tr>
<td>Surv 2005/179</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>t008</td>
<td>CC8</td>
<td>−</td>
<td>−</td>
<td>Skin</td>
</tr>
<tr>
<td>Surv 2003/651</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>051</td>
<td>CC8</td>
<td>−</td>
<td>−</td>
<td>Respiratory infection</td>
</tr>
</tbody>
</table>

Comparison of 2 strains & 3 antibiotics

Huge variability in activity among strains ...

Comparison of 7 strains & 5 antibiotics

Daptomycin and fluoroquinolones more potent… but again, high variability among strains

Importance of antibiotic concentration inside biofilms for activity

Activity in biofilm is correlated with antibiotic penetration

Biofilm matrix: what is it made of?

- Bacterial cell
- Enzyme
- Exopolysaccharide
- DNA
- Protein
- Water channel

Adjuvants acting on the matrix

Let’s try this amphipathic molecule … It looks like a detergent, doesn’t it?

Siala et al, Nature Communications 2016; 7:13286
Adjuvants acting on the matrix

But do you recognize this molecule?

Siala et al, Nature Communications 2016; 7:13286
Adjuvants acting on the matrix

Siala et al, Nature Communications 2016; 7:13286
Adjuvants acting on the matrix

Siala et al, Nature Communications 2016; 7:13286
delafloxacin +/- caspofungin on strain 2003/651

Siala et al, Nature Communications 2016; 7:13286
Caspofungin increases efficacy and relative potency of delafloxacin against a recalcitrant stain

*Siala et al, Nature Communications 2016; 7:13286*
Caspofungin-fluoroquinolone combinations

The combination works for two FQs and against several strains, but to different extents …

Siala et al, Nature Communications 2016; 7:13286
Caspofungin increases FQ potency within biofilms

The combination works for two FQs and against several strains, but to different extents …

Siala et al, Nature Communications 2016; 7:13286
Caspofungin increases fluoroquinolone activity \textit{in vitro} and \textit{in vivo}

\textit{Siala et al, Nature Communications 2016; 7:13286}
Caspofungin increases fluoroquinolone activity \textit{in vitro} and \textit{in vivo}

Caspofungin makes fluoroquinolones active at lower concentrations

\textit{Siala et al, Nature Communications 2016; 7:13286}
Caspofungin increases fluoroquinolone penetration

Siala et al, Nature Communications 2016; 7:13286
Effect of caspofungin on PNAG in biofilm matrix

Poly-N-acetylglucosamine

Siala et al, Nature Communications 2016; 7:13286
Effect of caspofungin on PNAG in biofilm matrix

Siala et al, Nature Communications 2016; 7:13286

CAS \Rightarrow \text{poly-N-acetylglucosamine content and polymerization in biofilms}
IacA and polysaccharide synthesis in *S. aureus*

*Ica A is involved in N-acetylglucosamine homopolymer synthesis*
Caspofungin, an unexpected IcaA inhibitor!

CAS inhibits IcaA and increases FQ potency in inverse proportion to icaA expression.

<table>
<thead>
<tr>
<th>strain</th>
<th>icaA expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATCC33591</td>
<td>1</td>
</tr>
<tr>
<td>2011S027</td>
<td>1.8 ± 0.5*</td>
</tr>
<tr>
<td>2003/1083</td>
<td>4.0 ± 0.6*</td>
</tr>
<tr>
<td>2009S025</td>
<td>2.5 ± 0.5*</td>
</tr>
<tr>
<td>2005/104</td>
<td>4.2 ± 0.4*</td>
</tr>
<tr>
<td>2005/179</td>
<td>6.0 ± 0.9*</td>
</tr>
<tr>
<td>2009S028</td>
<td>4.1 ± 0.2*</td>
</tr>
<tr>
<td>2003/651</td>
<td>16.3 ± 0.7*</td>
</tr>
</tbody>
</table>

*Siala et al, Nature Communications 2016; 7:13286*
The antifungal caspofungin as an inhibitor of polysaccharide synthesis

Candida albicans

Adapted from Arnold, Kucer’s 6th edition

Glucan synthase

Staphylococcus sp.


Mannoprotein polypeptide
Glucan polymers
Chitin

Candida albicans

Adapted from Arnold, Kucer’s 6th edition
Caspofungin, an unexpected IcaA inhibitor?

IcaA is homologous to glucan synthases (caspofungin target in fungi)

*Siala et al, Nature Communications 2016; 7:13286*
Caspofungin as a prototype for icaA inhibitors

Siala et al, Nature Communications 2016; 7:13286
Antibiofilm strategies under study in the lab …

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)

New clinical applications

1. Infections on medical devices

"Our endoscope is broken, but luckily nurse has her mobile phone camera - it's quite small..."

This is not the main reason for contamination...
Biofilms on endoscopes and cleaning procedures

uncleaned colonoscope

manual cleaning

paracetic acid

gluteraldehyde/alcool

removal of air/water noozle

### Importance of cleaning procedure

#### Summary of answers to the follow-up questionnaire for endoscope reprocessing procedures in 66 hospitals

<table>
<thead>
<tr>
<th>Characteristic and Recommendation</th>
<th>biofilm (n = 30)</th>
<th>No biofilm (n = 36)</th>
<th>Total (N = 66)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Daily surgical volume</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;5</td>
<td>70.0 (21/30)</td>
<td>83.3 (30/36)</td>
<td>78.8 (51/66)</td>
<td>.239</td>
</tr>
<tr>
<td>50-100</td>
<td>16.7 (5/30)</td>
<td>13.9 (5/36)</td>
<td>15.2 (10/66)</td>
<td></td>
</tr>
<tr>
<td>&gt;100</td>
<td>13.3 (4/30)</td>
<td>2.7 (1/36)</td>
<td>7.6 (5/66)</td>
<td></td>
</tr>
<tr>
<td>Proportion of manual cleaning</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Suctioning all channel</td>
<td>90.0 (27/30)</td>
<td>83.3 (30/36)</td>
<td>86.4 (57/66)</td>
<td>.670</td>
</tr>
<tr>
<td>Use of biofilm removal detergent</td>
<td>26.7 (8/30)</td>
<td>0 (0/36)</td>
<td>12.1 (8/66)</td>
<td>.003</td>
</tr>
<tr>
<td>Repeated use of detergent</td>
<td>63.3 (19/30)</td>
<td>91.7 (33/36)</td>
<td>78.8 (52/66)</td>
<td>.005</td>
</tr>
<tr>
<td>Sterile water used to rinse</td>
<td>60.0 (18/30)</td>
<td>61.1 (22/36)</td>
<td>60.6 (40/66)</td>
<td>.927</td>
</tr>
<tr>
<td>Alcohol dry</td>
<td>76.7 (23/30)</td>
<td>38.9 (14/36)</td>
<td>56.0 (37/66)</td>
<td>.002</td>
</tr>
</tbody>
</table>

**NOTE.** Values are percentages (compliance with recommendations for reprocessing or characteristic).

*Ren-Pei et al, Am J Infect Control 2014; 42:1203-6*
Efficacy of biofilm-removing detergents

In vitro evaluation of 12 detergent solutions against 15 biofilms from different species

High variability in capacity to act upon biofilms among detergents

Siala et al, unpublished
Efficacy of biofilm-removing detergents

UNTREATED

CLEANER A

GARUDA

CLEANER J

Siala et al, unpublished
## Efficacy of biofilm-removing detergents

### Ex vivo efficacy of GARUDA® for endoscope cleaning

<table>
<thead>
<tr>
<th>Hospital</th>
<th>Endoscope type</th>
<th>First microbiological control (in 100 ml)</th>
<th>Intensive cleaning and disinfection procedure</th>
<th>Second microbiological control (in 100 ml)</th>
<th>Garuda MD corrective cleaning and disinfection</th>
<th>Microbiological control after Garuda MD procedure (in 100 ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>French University Hospital</td>
<td>Echo-endoscope</td>
<td>&gt;150 CFU + Stenotrophomonas maltophilia</td>
<td>Yes (double 5 min manual cleaning + AER)</td>
<td>&gt; 150 CFU + Stenotrophomonas maltophilia</td>
<td>Garuda MD + AER</td>
<td>&lt; 1 CFU, Absence of Stenotrophomonas maltophilia</td>
</tr>
<tr>
<td>French University Hospital</td>
<td>Echo-endoscope</td>
<td>75 CFU + Pseudomonas aeruginosa + Streptococcus spp.</td>
<td>Yes (double 5 min manual cleaning + AER)</td>
<td>16 CFU + Stenotrophomonas maltophilia</td>
<td>Garuda MD + AER</td>
<td>0 CFU, Absence of Stenotrophomonas maltophilia</td>
</tr>
<tr>
<td>French University Hospital</td>
<td>Echo-endoscope</td>
<td>Return from maintenance / Not tested</td>
<td>Yes (double 5 min manual cleaning + AER)</td>
<td>&gt; 150 CFU + Stenotrophomonas maltophilia + Pseudomonas aeruginosa</td>
<td>Garuda MD + AER</td>
<td>0 CFU, Absence of Stenotrophomonas maltophilia and Pseudomonas aeruginosa</td>
</tr>
<tr>
<td>Belgian University Hospital</td>
<td>Gastroscope</td>
<td>&gt;300 CFU</td>
<td>Yes (No manual cleaning but longer AER cycle)</td>
<td>&gt; 300 CFU</td>
<td>Garuda MD + AER</td>
<td>0 CFU</td>
</tr>
<tr>
<td>Belgian Hospital #1</td>
<td>Gastroscope</td>
<td>&gt; 100 CFU + Pseudomonas aeruginosa</td>
<td>Yes (5 min manual cleaning + AER)</td>
<td>&gt; 100 CFU + Pseudomonas aeruginosa</td>
<td>Garuda MD + AER</td>
<td>0 CFU, Absence of Pseudomonas aeruginosa</td>
</tr>
<tr>
<td>Belgian Hospital #2</td>
<td>Duodenoscope</td>
<td>1,000 CFU + Pseudomonas aeruginosa</td>
<td>Yes (5 min manual cleaning + AER)</td>
<td>10,000 CFU + Pseudomonas aeruginosa</td>
<td>Garuda MD + AER</td>
<td>&lt; 20 CFU, Absence of Pseudomonas aeruginosa</td>
</tr>
<tr>
<td>Belgian Hospital #2</td>
<td>Duodenoscope</td>
<td>5,000 CFU + Pseudomonas aeruginosa</td>
<td>Yes (5 min manual cleaning + AER)</td>
<td>5,000 CFU + Pseudomonas aeruginosa</td>
<td>Garuda MD + AER</td>
<td>&lt; 20 CFU, Absence of Pseudomonas aeruginosa</td>
</tr>
</tbody>
</table>

AER: Automated Endoscope Reprocessors

GARUDA® removes most of remaining contamination

*Siala et al, unpublished*
New clinical applications

2. Infections on catheters

1. Attachment to unmodified polymer surface:
   van der Waal’s forces, hydrophobic interactions,
   SSP-1/SSP-2, AtlE, PSA/A, Bhp?

2. Attachment to polymer surface coated with
   extracellular matrix proteins: transcutaneous migration
   and/or hematogeneous seeding from distant site:
   AtlE, Fbe (SdrG), teichoic acid

3. Proliferation and accumulation in multilayered cell clusters:
   PIA, PS/A, AAP, Bhp?

Conditioning film:
   fibrin, fibrinogen, fibronectin, vitronectin,
   thrombospondin, von Willebrand factor

New clinical applications

Infections on catheters
Lock therapy and catheter-related infections

Totally implanted venous access catheters

- Closed system but accessible to colonisation
- Possibility to follow colonisation
  - in the chamber,
  - in the catheter,
  - the related infection

Antibiotic lock solution

Cancer chemotherapy
Parenteral nutrition
Cystic Fibrosis chemotherapy

Antibiotic lock therapy: current practice

Lebeaux et al, Lancet ID 2014; 14:146–159
Antibiotic lock therapy: current practice

50-80 % success

Uncomplicated BSI

50-80 % success

Uncomplicated BSI

> 50 % failure

VAN/cefazolin

Staphylococcus aureus

AmpB/CAS

Candida spp

Coagulase-negative staphylococci

Enterobacteria or Pseudomonas aeruginosa

Conservative treatment is possible, use ALT+ systemic antibiotics for 10–14 days; if TIVAP removed, use systemic antibiotics for 5–7 days

Conservative treatment is possible, use ALT+ systemic antibiotics for 10–14 days; if TIVAP removed, use systemic antibiotics for 7–14 days

Remove TIVAP; if no infective endocarditis or thrombophlebitis, start systemic antibiotics for 14 days, otherwise systemic antibiotics for ≥4 weeks

Remove TIVAP; start systemic antifungals; treatment should be continued for 14 days, after first negative blood culture; rule out dissemination with fundoscopy

If conservative treatment used, do control blood cultures after 3 days of treatment and 2–4 weeks after the end of treatment

If clinical or microbiological failure occurs, remove TIVAP

Lebeaux et al, Lancet ID 2014; 14:146–159
Antibiotic locks: clinical efficacy

Antibiotics compared with no antibiotics prior to long-term CVC insertion to prevent catheter-related infections

**Patient or population:** adults with a newly inserted long-term CVC who were at risk of neutropenia due to chemotherapy or disease

**Settings:** inpatient and outpatient

**Intervention:** intravenous antibiotics (vancomycin, teicoplanin or ceftazidime)

**Comparison:** placebo or no antibiotics

<table>
<thead>
<tr>
<th>Outcomes</th>
<th>Illustrative comparative risks* (95% CI)</th>
<th>Relative effect (95% CI)</th>
<th>No of Participants (studies)</th>
<th>Quality of the evidence (GRADE)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Assumed risk</td>
<td>Corresponding risk</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>200 per 1000</td>
<td>144 per 1000 (66 to 316)</td>
<td>RR 0.72 (0.33 to 1.58)</td>
<td>360 (5)</td>
<td>moderate</td>
</tr>
</tbody>
</table>

The difference between the comparison groups was not significant (P = 0.41). We downgraded this evidence to moderate due to substantial heterogeneity (I² = 52%) between studies.

*The basis for the **assumed risk** is the mean control group risk across studies. The **corresponding risk** (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).

CI: Confidence interval; RR: Risk ratio
## Anti-coagulant + antibiotic locks: clinical efficacy

**Antibiotic and heparin solution compared with a heparin only solution for flushing or locking long-term CVCs to prevent Gram positive catheter-related sepsis**

**Patient or population:** adults and children with a newly inserted long-term CVC who were at risk of neutropenia due to chemotherapy or disease

**Settings:** inpatient and outpatient

**Intervention:** antibiotic (vancomycin, vancomycin and amikacin, or taurolidine) plus heparin solution

**Comparison:** heparin only solution

<table>
<thead>
<tr>
<th>Outcomes</th>
<th>Illustrative comparative risks* (95% CI)</th>
<th>Relative effect (95% CI)</th>
<th>No of Participants (studies)</th>
<th>Quality of the evidence (GRADE)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Assumed risk</strong></td>
<td><strong>Corresponding risk</strong></td>
<td>RR 0.47 (0.28 to 0.80)</td>
<td>468 (6)</td>
<td>☀ ☀ ☀ ☀ moderate</td>
<td>Data consistent across included studies; $I^2 = 0%$, $P = 0.005$. For an assumed risk of 15%, the NNT = 12 (9 to 33). We downgraded this evidence to moderate as the sample was clinically heterogeneous</td>
</tr>
<tr>
<td>Catheter-related sepsis</td>
<td>200 per 1000</td>
<td>94 per 1000 (56 to 160)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*The basis for the assumed risk is the mean control group risk across included studies. The corresponding risk (and its 95% confidence interval) is based on the assumed risk in the comparison group and the relative effect of the intervention (and its 95% CI). CI: Confidence interval; RR: Risk ratio; NNT: number needed to treat.
Lock therapy in the lab: screening of antibiotics

Results of treatment of experimental MRSA catheter-related sepsis

<table>
<thead>
<tr>
<th>Treatment</th>
<th>MRSA 7 strain</th>
<th>MRSA 16 strain</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>negative cultures/total</td>
<td>log_{10} total cfu (SD)</td>
</tr>
<tr>
<td>Control</td>
<td>0/15</td>
<td>5.90 (1.13)</td>
</tr>
<tr>
<td>Linezolid</td>
<td>0/10</td>
<td>5.13 (0.94)</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>0/13</td>
<td>5.11 (1.05)</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>4/10</td>
<td>2.19 (1.78)</td>
</tr>
</tbody>
</table>

\(^{a}P<0.05\) versus control.
\(^{b}P<0.01\) versus linezolid and vancomycin.


Aminoglycosides + EDTA

Genta + EDTA : highly synergistic combination

Modulation of gentamicin activity: L-Arginine

In vitro synergism

L-Arginine improves Genta activity by increasing pH

Modulation of gentamicin activity: L-Arg

In vivo synergism

In vivo, some of the L-Arg effects are pH independent

Grafting non-biocidal anti-adhesion molec. on catheters

**Coating prevents bacterial adhesion**

**Chauhan et al. J. Infect. Dis. 2014; 210:1347–1356.**
Grafting non-biocidal anti-adhesion molec. on catheters

adherence colonization at day 5

Coating prevents bacterial adhesion and further colonization

3. Orthopedic infections

biofilm observed in electron microscopy on a steel component of an Ilizarov device obtained from a patient with clinical infection (S. aureus)

Bartoszewicz et al; Orthopedia Traumatologia Rehabilitacja 2007; 9:310-8
Evidence for biofilm in orthopedic infections

Increased risk of treatment failure for biofilmogenic
*S. epidermidis* in Device-Related Osteomyelitis of the Lower Extremity
in Human Patients

Descriptive and Univariable Analysis of Prognostic Factors for “cure” in the Lower Extremity Cohort

<table>
<thead>
<tr>
<th>Prognostic Factor</th>
<th>Cured</th>
<th></th>
<th>Univariable Regression Model for “cure”</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Odds Ratio for “cure”</td>
<td>95%-Confidence Interval</td>
<td>p-Value</td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>19 (25.7)</td>
<td>55 (74.3)</td>
<td>0.53</td>
<td>(0.26; 1.07)</td>
<td>0.076</td>
<td></td>
</tr>
<tr>
<td><strong>Biofilm formation</strong>&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non</td>
<td>4 (16.0)</td>
<td>21 (84.0)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weak</td>
<td>7 (24.1)</td>
<td>22 (75.9)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Marked&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8 (40.0)</td>
<td>12 (60.0)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Evidence for biofilm in orthopedic infections

Persistent isolates of *S. aureus* are higher biofilms producers ….

Table 1. Characteristics of the patients and isolates.

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Sex, age (year)</th>
<th>Site of infection</th>
<th>Duration of symptoms (days)$^a$</th>
<th>Surgical treatment</th>
<th>Duration of antibiotic therapy (days)</th>
<th>Time to failure or relapse (days)$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M, 26</td>
<td>Tibia osteosynthesis material</td>
<td>12</td>
<td>Material removed</td>
<td>82</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>M, 80</td>
<td>Total knee arthroplasty</td>
<td>3</td>
<td>Irrigation and debridement</td>
<td>191</td>
<td>10</td>
</tr>
<tr>
<td>3</td>
<td>F, 82</td>
<td>Total hip arthroplasty</td>
<td>3</td>
<td>Irrigation and debridement</td>
<td>98</td>
<td>36</td>
</tr>
</tbody>
</table>

Biomaterials for antibiotic delivery

Available prophylactic biomaterials vehicles:

a. Collagen (hypersensitivity, poor handling)
b-c: PMMA \[^{\text{methylmetacrylate}}\] beads or spacers (non degradable)
d. PDLLA \[^{\text{poly-D,\text{L-lactide}}\}]\) (acidic degradation products)
e. Calcium sulfates (osteocoendeuctive)

Images: Dr. Mario Morgenstern BGU Murnau, Germany; Dr Menendez, Jobe Orthopaedic Clinic, Los Angeles, CA
Biomaterials for antibiotic delivery

Variable antibiotic release from commercial cements

*ter Boo et al, Biomaterials 2015, 52:113-25.*
Antibiotic-loaded bone cements: clinical experience

<table>
<thead>
<tr>
<th>Study by Arthroplasty Site</th>
<th>Study Period</th>
<th>Patients, No./Joints, No.</th>
<th>Spacer Antibiotic Content (Dose, g/40 g Cement)</th>
<th>Infection Eradication Ratea By Review</th>
<th>As Reported by Authors</th>
<th>Deathsb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Knee</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[43]</td>
<td>Not reported</td>
<td>12/12</td>
<td>Tobramycin (4.8) + vancomycin (4)</td>
<td>12/12 (100)</td>
<td>12/12 (100)</td>
<td>0</td>
</tr>
<tr>
<td>[17]c</td>
<td>1995–2002</td>
<td>29/31</td>
<td>Tobramycin (4.6) + vancomycin (4)</td>
<td>25/31 (81)</td>
<td>29/31 (93)</td>
<td>0</td>
</tr>
<tr>
<td>[13]d</td>
<td>1997–1999</td>
<td>58/58</td>
<td>Tobramycin (3.6) + vancomycin (1.5)</td>
<td>48/58 (83)</td>
<td>45/47 (96)</td>
<td>NAe</td>
</tr>
<tr>
<td>[44]</td>
<td>1998–2001</td>
<td>24/24</td>
<td>Tobramycin (2.4) + vancomycin (1)</td>
<td>22/24 (92)</td>
<td>22/24 (92)</td>
<td>0</td>
</tr>
<tr>
<td>[45]</td>
<td>1996–2001</td>
<td>28/28</td>
<td>Tobramycin (1.2) or gentamicin (1) + vancomycin (1)</td>
<td>25/28 (89)</td>
<td>25/28 (89)</td>
<td>0</td>
</tr>
<tr>
<td>[46]</td>
<td>2000–2005</td>
<td>36/36</td>
<td>Piperacillin-tazobactam (4.5) + vancomycin (2) + erythromycin (1)</td>
<td>32/36 (89)</td>
<td>32/36 (89)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[18]</td>
<td>1989–2001</td>
<td>50/50</td>
<td>Tobramycin (4.8)</td>
<td>44/50 (88)</td>
<td>44/50 (88)</td>
<td>NA</td>
</tr>
<tr>
<td>[22]</td>
<td>1994–2002</td>
<td>44/44</td>
<td>Tobramycin (4.8)</td>
<td>43/44 (98)</td>
<td>43/44 (98)</td>
<td>0</td>
</tr>
<tr>
<td>[14]d</td>
<td>1986–1999</td>
<td>40/40</td>
<td>Tobramycin (1.2)</td>
<td>36/40 (90)</td>
<td>36/40 (90)</td>
<td>0</td>
</tr>
<tr>
<td>[19]</td>
<td>1989–1993</td>
<td>69/69</td>
<td>Tobramycin (1)</td>
<td>60/69 (87)</td>
<td>61/69 (88)</td>
<td>0</td>
</tr>
<tr>
<td>Hip</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[17]c</td>
<td>1995–2002</td>
<td>16/23</td>
<td>Tobramycin (4.6) + vancomycin (4)</td>
<td>18/23 (78)</td>
<td>22/23 (96)</td>
<td>0</td>
</tr>
<tr>
<td>[48]</td>
<td>Not reported</td>
<td>12/12</td>
<td>Tobramycin (3.6) + vancomycin (1)</td>
<td>12/12 (100)</td>
<td>12/12 (100)</td>
<td>0</td>
</tr>
<tr>
<td>[12]d</td>
<td>1998–2001</td>
<td>22/22</td>
<td>Tobramycin (2.4) + vancomycin (1)</td>
<td>20/22 (90)</td>
<td>20/20 (100)</td>
<td>2 (9)</td>
</tr>
<tr>
<td>[10]d</td>
<td>1993–1997</td>
<td>24/24</td>
<td>Gentamicin (1) + vancomycin (1) + cepotaxime (1)</td>
<td>21/24 (83)</td>
<td>21/22 (95)</td>
<td>2 (8)</td>
</tr>
<tr>
<td>[16]d,f</td>
<td>1998–2003</td>
<td>43/44</td>
<td>Gentamicin (0.25) + vancomycin (2)</td>
<td>35/44 (80)</td>
<td>38/41 (93)</td>
<td>3 (7)</td>
</tr>
<tr>
<td>[23]h</td>
<td>2001–2006</td>
<td>40/40</td>
<td>Gentamicin (0.76)</td>
<td>38/40 (95)</td>
<td>39/40 (97.5)</td>
<td>0</td>
</tr>
</tbody>
</table>

Iarikov et al., Clin Infect Dis 2012; 55:1474-80
New developments: an example

Thermoresponsive Hyaluronan hydrogel

Rapid release of gentamicin from the gel; low serum levels

Ter Boo et al., Acta Biomaterialia 2016; 43:185–194
New developments: an example

Thermoresponse Hyaluronic hydrogel

Fig. 1. Intra-operative image before (A) and after (B) application of the gentamicin-loaded HApN hydrogel within the surgical field. The HApN hydrogel (white color) fills the surgical field and turns from a sol to a gel state upon contact with the tissue.

Genta-loaded hydrogel reduces infection

Ter Boo et al., Acta Biomaterialia 2016; 43:185–194
Take home messages

- Antibiotic activity poor against biofilms due to PK/PD issues
- Combinations with adjuvants effective in animal models
- Prevention easier than cure …
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Patrick Van Dijck
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