Intracellular Bacterial Infections: Why Are Antibiotics Poorly Efficient and Can We Do Something About it?

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* with slides borrowed from Françoise Van Bambeke and Frederic Peyrusson
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• Research grants
  – Theravance, Astellas, Targanta, Cerexa/Forest, AstraZeneca, Bayer, GSK, Trius, Rib-X, Eumedica, Debiopharm
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  – Bayer, GSK, Sanofi, Johnson & Johnson, OM-Pharma

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  – European Committee for Antimicrobial Susceptibility Testing [EUCAST] (General Assembly and steering committee (2010-2012))
  – European Medicines Agency (external ad-hoc expert)
  – US National Institutes of Health (grant reviewing)
  – Drive-AB [Driving reinvestment in R&D and responsible use for antibiotics] (governance)

Slides: http://www.facm.ucl.ac.be → Lectures
Belgium
Belgium

10 millions inhabitants …

10 Nobel prizes (10/850)

• Peace
  - Institute of International Law, Ghent (1904)
  - Auguste Beernaert (1909)
  - Henri Lafontaine (1913)
  - Father Dominique Pire (1958)

• Literature
  - Maurice Maeterlinck, Ghent (1911)

• Medicine
  - Jules Bordet, Brussels (1919)
  - Corneille Heymans, Ghent (1938)
  - Christian de Duve, Louvain (1974)
  - Albert Claude, Brussels (1974)

• Chemistry
  - Ilya Prigogyne, Brussels (1977)

• Physics
  - François Englert, Brussels (2013)
The Catholic University of Louvain in brief (1 of 4)

- originally founded in 1425 in the city of Louvain (in French and English; known as Leuven in Flemish)
The Catholic University of Louvain in brief (2 of 4)

- It was one of the major University of the so-called "Low Countries" in the 1500 – 1800 period, with famous scholars and discoverers (Vesalius for anatomy, Erasmus for philosophy, …). Teaching was in Latin, Greek, and Hebrew (College of the 3 languages…)

The University in the 1500's

Erasmus

Vesalius
The Catholic University of Louvain in brief (3 of 4)

- In the 19th century, teaching was in French but in the early 1900's, a Flemish-speaking section was opened. Courses were given in both languages, attracting many students and celebrities…

Prof. G. Lemaitre, professor of Physics and Mathematics at the University who, in the 1930's, made the first suggestion of the continuous expansion of the Universe ("big bang") (here in conversation with A. Einstein)

Professor C. de Duve, Professor of Biochemistry, obtained the Nobel Prize (Physiology and Medicine) in 1974 for his work on intracellular organelles (lysosomes, peroxisomes…)

(here in front of a centrifuge)

- in 1968, the University was divided into
  - a French-speaking Université catholique de Louvain
  - a Flemish-speaking Katholieke Universiteit Leuven…
The Catholic University of Louvain in brief (4 of 4)

- The Flemish-speaking *Katholieke Universiteit Leuven* has remained in Louvain (Leuven) and is named in English "Catholic Universiteit Leuven".
- The French-speaking *Université catholique de Louvain* has moved about 25 km South in a place called "Louvain-la-Neuve, with the "Health Sciences Sector" located in Brussels (Woluwe).

Together, the two Universities have about **55,000 students**

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Université catholique de Louvain  
http://www.uclouvain.be

Katholieke Universiteit Leuven  
http://www.kuleuven.be
The Faith and Reason of Father George Lemaître

by Joseph R. Laracy

The Big Bang hypothesis is widely known in popular thought as the best explanation for how the universe came to be. However, very few people know that a Catholic priest formulated this theory in the late 1920s. Reverend Monsignor Georges Lemaître, a Belgian scientist, challenged the conventional thinking of his colleagues, including Albert Einstein, and rejected the static universe hypothesis for a dynamic model. In the course of carrying out his research, he confronted illogical thinking that pitted faith against reason, and science against the Church. His legacy extends beyond cosmology, to the nature of truth itself.
Notre Dame and the "Big Bang"

The Faith and Reason of Father George Lemaître

by Joseph R. Laracy

The Big Bang hypothesis is the best explanation for the age of the universe, but very few people know that the ideas behind it were put forth in the late 1920s. Reverend Georges Lemaître, a Belgian scientist, challenged the prevailing scientific consensus and appealed to his faith. His colleagues, including Albert Einstein, were not convinced of the Big Bang hypothesis for several years. After Lemaître's death in 1976, his legacy extends beyond his hometown of Ghent to his great contributions to cosmology.

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Mar. 1931.


(Translated by permission from “Annales de la Société scientifique de Bruxelles,” Tome XLVII, série A, première partie.)

https://dx.doi.org/10.1093%2Fmnras%2F91.5.483

In 1936, Father John O'Hare, the president of the University of Notre Dame, hired Father Lemaître as a visiting professor. During that year, his course on cosmology was not only attended by graduate students, but also faculty members in the physics and mathematics departments.

So, now, our small bang...

**S. aureus in THP-1 macrophages**

[Image: Van Bambeke & Tulkens unpublished]

**S. aureus in human osteoblasts**


**S. aureus in and released from neutrophils in a mouse osteomyelitis model**


*intracellular S. aureus*
And more bangs...

Fig. 1. Electron micrographs of different types of infected host cells. Adherence and uptake of *S. aureus* in epithelial A549 cells (A). Intracellular location of *S. aureus* after infection of primary osteoblasts (B). Dividing figure of *S. aureus* within an intracellular phagosome (C) and intracellular bacterial degradation (D) 24 h after infection of endothelial cells (HUVEC).
Why do we wish to look at intracellular activity of antibiotics?

• Beyond truly obligate intracellular parasites (e.g., *Legionella*, *Chlamydia*, *Mycobacteria*, …many more "common" bacteria are facultative (e.g. *Listeria*) or occasional (e.g. *Staphylococci*, *Pseudomonas*) intracellular parasites …

• These bacteria form a *reservoir* from where bacteria may escape causing *relapses* and *recurrences* of the infection…

• Natural defenses often restrict their growth and decrease their persistence, but not always…

• You may need to help host defenses with *antibiotics*
Are antibiotics active at all in cells?  

Control (no antibiotic) 2

Oxacillin 63 mg/L  
(= C_{max} and 500 x MIC)

Oritavancin 25 mg/L  
(= C_{max} and 100 x MIC)

2 gentamicin added at 1 x MIC to prevent extracellular growth
Intracellular activity of antibiotics

• What has been know for long about pharmacokinetics …

• What has surprised us …

• Adding pharmacodynamics …

• A renewed model ?
Intracellular activity of antibiotics

• What has been known for long about pharmacokinetics…

• What has surprised us …

• Adding pharmacodynamics  …

• A renewed model ?
A simple view in 1991


**Figure 1:** Pharmacokinetic and pharmacodynamic parameters involved in the activity of antimicrobial drugs against intracellular microorganisms.
Which antibiotics accumulate in cells?

- beta-lactams: ≤ 1x
- aminoglycosides: <1 to 2x
- ansamycins: 2-3x
- tetracyclines: 2-4x
- fluoroquinolones: 5 - 20x
- macrolides: 4 to > 100x *
- glycopeptides: 1 to 400x !! **

* azithromycin, ketolides
** oritavancin
How do antibiotics penetrate in cells?

1. diffusion

- macrolides
- fluoroquinolones
- tetracyclines
- ansamycines
- $\beta$-lactams,
- ...
How do antibiotics penetrate in cells?

2. carrier-mediated influx

- specific structure
- (some energy-dependent)
- saturable
- competition by analogues

highly variable from cell type to another
How do antibiotics penetrate in cells?

3. pinocytosis

- aminoglycosides
- glycopeptides
How do antibiotics penetrate in cells?

**receptor-mediated pinocytosis in kidney cortex**

**Binding to megalin and acidic phospholipids**

- Silverblatt & Kuehn C. Kidney Int. 1979;15:335-45 - PMID: 513493
- Sastrasinh et al. J Pharmacol Exp Ther 1982;222:350-8 - PMID: 7097385
- Giuliano et al. J Pharmacol Exp Ther 1986;236:470-5 - PMID: 3944766
How do antibiotics penetrate in cells?

membrane binding and uptake of lipoglycopeptides

But once in cells, where are the drugs?
Subcellular localization: a quick answer?

- Cytosol:
  - fluoroquinolones
  - beta-lactams
  - ansamycins
  - macrolides (1/3)

- Endosomes

- Lysosomes:
  - macrolides (2/3)
  - aminoglycosides

- Phagosomes?

- Phagolysosomes?
So, what we know in a nutshell ...

<table>
<thead>
<tr>
<th>Pharmacocchemical class</th>
<th>Antibiotic</th>
<th>Accumulation level at equilibrium (C_C)/C_e</th>
<th>Cellular concentration at equilibrium (mg/l)</th>
<th>Time to equilibrium</th>
<th>Predominant subcellular localization</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-Lactams</td>
<td>All</td>
<td>&lt; 1</td>
<td>~ 20 to 50</td>
<td>Fast</td>
<td>Cytosol</td>
</tr>
<tr>
<td>Macrolides</td>
<td>Erythromycin</td>
<td>4 to 10</td>
<td>~ 40 to 150</td>
<td>Moderate (a few hours)</td>
<td>2/3 Lysosomes 1/3 Cytosol</td>
</tr>
<tr>
<td></td>
<td>Clarithromycin</td>
<td>10 to 50</td>
<td>~ 20 to 400</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Roxithromycin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Telithromycin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Azithromycin</td>
<td>40 to 300</td>
<td>~ 16 to 120</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fluoroquinolones</td>
<td>Ciprofloxacin</td>
<td>4 to 10</td>
<td>~ 16 to 40</td>
<td>Fast (&lt; 1 h) to very fast (&lt; 5 min)</td>
<td>Cytosol</td>
</tr>
<tr>
<td></td>
<td>Levofloxacin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Grepafloxacin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Moxifloxacin</td>
<td>10 to 20</td>
<td>~ 40 to 80</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Garenoxacin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Gemifloxacin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aminoglycosides</td>
<td>All</td>
<td>2 to 4 (after several days)</td>
<td>~ 40 to 80</td>
<td>Slow (several days)</td>
<td>Lysosomes</td>
</tr>
<tr>
<td>Lincosamides</td>
<td>Clindamycin</td>
<td>5 to 20</td>
<td>~ 50 to 200</td>
<td>Fast</td>
<td>Unknown</td>
</tr>
<tr>
<td></td>
<td>Lincomycin</td>
<td>1 to 4</td>
<td>~ 15 to 60</td>
<td></td>
<td>Unknown</td>
</tr>
<tr>
<td>Tetracyclines</td>
<td>Probably all</td>
<td>1 to 4</td>
<td>~ 2 to 12</td>
<td>Unknown</td>
<td>Unknown</td>
</tr>
<tr>
<td>Ansamycins (rifamycins)</td>
<td>Rifampin</td>
<td>2 to 10</td>
<td>~ 36 to 180</td>
<td>Unknown</td>
<td>Unknown</td>
</tr>
<tr>
<td></td>
<td>Rifapentine</td>
<td>60 to 80</td>
<td>~ 1200 to 1600</td>
<td>Unknown</td>
<td>Unknown</td>
</tr>
<tr>
<td>Glycopeptides</td>
<td>Vancomycin</td>
<td>8 (after 24 h)</td>
<td>~ 400</td>
<td>Slow (several hours)</td>
<td>Lysosomes (in kidney)</td>
</tr>
<tr>
<td></td>
<td>Teicoplanin</td>
<td>60</td>
<td>~ 6000</td>
<td></td>
<td>Unknown</td>
</tr>
<tr>
<td></td>
<td>Oritavancin</td>
<td>150 to 300 (after 24 h)</td>
<td>~ 3750 to 7500</td>
<td></td>
<td>Lysosomes</td>
</tr>
<tr>
<td></td>
<td>Telavancin</td>
<td>50 (after 24 h)</td>
<td>~ 4500</td>
<td></td>
<td>Lysosomes</td>
</tr>
<tr>
<td>Oxazolidinones</td>
<td>Linezolid</td>
<td>~ 1</td>
<td>~ 20</td>
<td></td>
<td>Unknown</td>
</tr>
</tbody>
</table>

Adapted from Van Bambeke et al., Curr Opin Drug Discov Devel 2006;9:218-230 – PMID: 16566292
But where does this lead us for activity?

* taken from a slide presented at ECCMID in 2002
Intracellular activity of antibiotics

• What has been known for long about pharmacokinetics...

• What has surprised us ...

• Adding pharmacodynamics ...

• A renewed model?
Intracellular activity is not directly correlated to accumulation

Adapted from Van Bambeke et al., Curr Opin Drug Discov Devel 2006;9:218-230 – PMID: 16566292
Intracellular activity is not directly correlated to accumulation

\[ \text{Listeria monocytogenes} \]

\[ \text{Staphylococcus aureus} \]

- AMP = ampicillin
- AZM = azithromycin
- CIP = ciprofloxacin
- ETP = ertapenem
- GEN = gentamicin
- GRN = garenoxacin
- LNZ = linezolid
- LVX = levofloxacin
- MEM = meropenem
- MXF = moxifloxacin
- NAF = nafcillin
- ORI = oritavancin
- OXA = oxacillin
- PEN V = penicillin V
- RIF = rifampicin
- TEC = teicoplanin
- TEL = telithromycin
- VAN = vancomycin

Adapted from Van Bambeke et al., Curr Opin Drug Discov Devel 2006;9:218-230 – PMID: 16566292
Intracellular activity is not directly correlated to accumulation

---

**Listeria monocytogenes**

- **Δ log CFU from time 0**
  - **Log cellular concentration** (concentration in mg/l)

**Staphylococcus aureus**

- **Δ log CFU from time 0**
  - **Log cellular concentration** (concentration in mg/l)

---

AMP = ampicillin; AZM = azithromycin; CIP = ciprofloxacin; ETP = ertapenem; GEN = gentamicin; GRN = garenoxacin; LNZ = linezolid; LVX = levofloxacin; MEM = meropenem; MXF = moxifloxacin; NAF = nafcillin; ORI = oritavancin; OXA = oxacillin; PEN V = penicillin V; RIF = rifampicin; TEC = teicoplanin; TEL = telithromycin; VAN = vancomycin

Adapted from Van Bambeke et al., Curr Opin Drug Discov Devel 2006;9:218-230 – PMID: 16566292
Thus, there is now an obvious conclusion

"Accumulation only" may not be the key property

One size does not fill all

Each class of antibiotic / bacteria combination may need to be examined separately
Subcellular bioavailability of antibiotics?

High  Fair  Nil

FQ / oxazolidinones / β-lactams  ML / AG
Subcellular bioavailability of antibiotics?

Fluoroquinolones, β-lactams, oxazolidinones, ... may move easily across membranes
Conversely, poorly diffusible antibiotics (aminoglycosides, oritavancin, e.g.) or subjected to proton-trapping sequestration (macrolides, e.g.), may remained confined where they are...
Intracellular activity of antibiotics

- What has been know for long about pharmacokinetics...

- What has surprised us ...

- Adding pharmacodynamics ...

- A renewed model?
24h pharmacodynamic dose-effect model

1. Cell exposure to a wide range of extracellular concentrations of the antibiotic

- **Opsonization** (45’, 37°C)
  - 9 mL RPMI +
  - 1 mL human serum

- **Phagocytosis** (1 h)
  - 500,000 THP-1 cells/mL
  - 4 cfu/cell (MOI = 4)

- **Extracellular Wash**
  - GEN 50 µg/mL
    - (45 min)
  - Typical post-phagocytosis inoculum:
    - 5 to 7x10⁵ CFU/mg prot.

- **Incubation (with ATB)**
  - (T0, T24 h)

- **Typical post-phagocytosis inoculum:**
  - Cell washing, collection, and lysis
  - Cell-associated CFUs counting
  - Cell Protein content determination

This example is for *S. aureus*. Similar design for other bacteria

Interpretation of the results of the 24h dose-effect model

2. Analysis of the response

![Graph showing Log10 of extracellular concentration (× MIC) vs. ∆ Log10 cfu (24 h – 0 h)]

- **E_{min}**: cfu increase (in log_{10} units) at 24 h from the corresponding initial inoculum as extrapolated for an infinitely low antibiotic concentration.

- **E_{max}**: cfu decrease (in log_{10} units) at 24 h from the corresponding initial inoculum as extrapolated from infinitely large antibiotic concentration.

- **C_{stat}**: extracellular concentration resulting in no apparent bacterial growth (number of cfu identical to the initial inoculum).

Interpretation of the results of the 24h dose-effect model

2. the analysis of the response

\[ \Delta \log_{10} \text{cfu (24 h – 0 h)} \]

**E_{min}:** cfu increase (in log_{10} units) at 24 h from the corresponding initial inoculum as extrapolated for an infinitely low antibiotic concentration

**C_{stat}:** extracellular concentration resulting in no apparent bacterial growth (number of cfu identical to the initial inoculum)

**E_{max}:** cfu decrease (in log_{10} units) at 24 h from the corresponding initial inoculum

Are intracellular and extracellular activities equal?

S. aureus model (ATCC25223)


Compare the extracellular and the intracellular $E_{\text{max}}$.
Antibiotics have a much lower intracellular $E_{max}$...

$S.\ aureus$ model
(ATCC33591 [MRSA])

**Figure 1.** Concentration-dependent activities of four antistaphylococcal antibiotics against extracellular (MHB broth pH 7.4 (a)) and intracellular (THP-1 monocytes (b)) forms of $S.\ aureus$ strain ATCC 33591 (MRSA). For these experiments, broths or infected cells were incubated for 24 h in the presence of increasing concentrations of antibiotic (total drug; abscissa). The ordinates show the change in the number of colony-forming units (CFU) per mL of broth or per mg or cell protein (THP-1). Note that because of the marked difference in the amplitude of the broth versus bacteria in THP-1 cells, the scale extends from $-6$ to $4$ in panel (a) and from $-1$ to $3$ in panel (b) showing the zero value (no apparent change from the initial, post-phagocytosis inoculum). All values are the means of three replicates. SD bars are smaller than the size of the symbols. The lowest limit of detection corresponds to a CFU count of $10^0$ in broth or to the original inoculum. The grey zone shows the range of maximal serum concentrations observed in healthy humans based on the following reported $C_{max}$ values: ceftaroline, 21 mg/L; vancomycin, 20–50 mg/L; daptomycin, 5 mg/L.

Antibiotics have a much lower intracellular $E_{\text{max}}$ ... but also often a similar $C_s$ than in broth

Van Bambeke & Tulkens, ASM Microbe 2016 – poster SARTURDAY 571 – Session 188
Numerical values...

<table>
<thead>
<tr>
<th>antibiotic</th>
<th>strain</th>
<th>$E_{\text{max}}$ (log10 CFU decr.)</th>
<th>$C_s$ (multiple of MIC)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>broth</td>
<td>intracellular</td>
</tr>
<tr>
<td><strong>S. aureus</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ceftaroline</td>
<td>ATCC33591</td>
<td>-5.3</td>
<td>-0.56</td>
</tr>
<tr>
<td></td>
<td>multiple strains</td>
<td>-5.1</td>
<td>-0.58</td>
</tr>
<tr>
<td>daptomycin</td>
<td>ATCC33591</td>
<td>-5.1</td>
<td>-0.99</td>
</tr>
<tr>
<td>GSK1322322 a</td>
<td>ATCC25923</td>
<td>-5.5</td>
<td>-0.48</td>
</tr>
<tr>
<td>moxifloxacin</td>
<td>ATCC25923</td>
<td>-4.3</td>
<td>-2.7</td>
</tr>
<tr>
<td><strong>P. aeruginosa</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ceftazidime</td>
<td>PAO1</td>
<td>-5.1</td>
<td>-1.3</td>
</tr>
<tr>
<td>meropenem</td>
<td>PAO1</td>
<td>-6.0</td>
<td>-3.0</td>
</tr>
<tr>
<td>colistin</td>
<td>PAO1</td>
<td>-5.4</td>
<td>-1.0</td>
</tr>
<tr>
<td>ciprofloxacin</td>
<td>PAO1</td>
<td>-5.2</td>
<td>-2.6</td>
</tr>
<tr>
<td>RX-P853 b</td>
<td>multiple strains</td>
<td>-5.1</td>
<td>-2.4</td>
</tr>
</tbody>
</table>

a a novel peptide deformylase inhibitor with activity against multi-resistant *S. aureus*
b a novel inhibitor of bacterial protein synthesis acting a the translation step with broad spectrum activity

Van Bambike & Tulkens, ASM Microbe 2016 – poster SARTURDAY 571 – Session 188
A few more comparisons of $E_{\text{max}}$…

<table>
<thead>
<tr>
<th>Antibiotic Class</th>
<th>Molecule *</th>
<th>Emax ($\Delta \log_{10}$CFU at 24h)</th>
<th>Extracellular **</th>
<th>intracellular</th>
</tr>
</thead>
<tbody>
<tr>
<td>beta-lactams</td>
<td>oxacillin 1</td>
<td>-3.1</td>
<td>-1.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ceftaroline 2</td>
<td>-5.4</td>
<td>-0.6</td>
<td></td>
</tr>
<tr>
<td>lipopeptides</td>
<td>daptomycin 2</td>
<td>-5.1</td>
<td>-1.0</td>
<td></td>
</tr>
<tr>
<td>fluoroquinolones</td>
<td>moxifloxacin 4</td>
<td>-4.8</td>
<td>-2.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ciprofloxacin 5</td>
<td>-4.9</td>
<td>-1.6</td>
<td></td>
</tr>
<tr>
<td>pyrrolocytosines</td>
<td>RX-P873 6</td>
<td>-4.2</td>
<td>-0.7</td>
<td></td>
</tr>
<tr>
<td>peptides (defensins)</td>
<td>NZ2114 7</td>
<td>-4.1</td>
<td>-1.5</td>
<td></td>
</tr>
<tr>
<td>deformylase inhibitors</td>
<td>GSK1322322 3</td>
<td>-4.8</td>
<td>-0.4</td>
<td></td>
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<tr>
<td>glycopeptides</td>
<td>vancomycin 2</td>
<td>-5.1</td>
<td>-0.6</td>
<td></td>
</tr>
<tr>
<td>lipoglycopeptides</td>
<td>oritavancin 1</td>
<td>-5.5</td>
<td>-3.1</td>
<td></td>
</tr>
<tr>
<td>oxazolidinones</td>
<td>linezolid 2</td>
<td>-2.9</td>
<td>-0.3</td>
<td></td>
</tr>
</tbody>
</table>

* all molecules but linezolid are highly bactericidal by conventional MBC/MIC measurements
** limit of detection: $-5.5 \log_{10}$ units

Reminder: $E_{\text{max}}$ is the maximal reduction of the initial inoculum for an infinitely large drug concentration
A few more comparisons of $E_{\text{max}}$…

<table>
<thead>
<tr>
<th>Antibiotic Class</th>
<th>Molecule *</th>
<th>$E_{\text{max}}$ ($\Delta \log_{10} \text{CFU at 24h}$)</th>
<th>Extracellular **</th>
<th>Intracellular</th>
</tr>
</thead>
<tbody>
<tr>
<td>beta-lactams</td>
<td>oxacillin $^1$</td>
<td>-3.1</td>
<td>-1.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ceftaroline $^2$</td>
<td>-5.4</td>
<td>-0.6</td>
<td></td>
</tr>
<tr>
<td>lipopeptides</td>
<td>daptomycin $^2$</td>
<td>-5.1</td>
<td>-1.0</td>
<td></td>
</tr>
<tr>
<td>fluoroquinolones</td>
<td>oxazofloxacin</td>
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<td>-2.0</td>
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<tr>
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</tbody>
</table>

* all molecules but linezolid are highly bactericidal by conventional MBC/MIC measurements
** limit of detection: $-5.5 \log_{10}$ units

References:

\[a\] Reminder: $E_{\text{max}}$ is the maximal reduction of the initial inoculum for an infinitely large drug concentration.
Some antibiotics are better…

<table>
<thead>
<tr>
<th>Antibiotic Class</th>
<th>Molecule *</th>
<th>Emax (Δlog_{10}CFU at 24h)</th>
<th>Extracellular **</th>
<th>intracellular</th>
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<tbody>
<tr>
<td>beta-lactams</td>
<td>oxacillin ¹</td>
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<td></td>
<td>ceftaroline ²</td>
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<td>ciprofloxacin ⁵</td>
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* all molecules but linezolid are highly bactericidal by conventional MBC/MIC measurements

** limit of detection: -5.5 log10 units


Reminder: \( E_{max} \) is the maximal reduction of the initial inoculum for an infinitely large drug concentration
Intracellular activity of antibiotics

• What has been know for long about pharmacokinetics…

• What has surprised us …

• Adding pharmacodynamics …

• A renewed model ?
1. Penetration

This is obvious:
no penetration = no activity
ex.: aminoglycosides in short term exposures
The seven pillars of intracellular activity?

1. Penetration
2. No efflux

Also obvious:
- efflux decreases the intracellular concentration
  ex.: fluoroquinolones (MRP4), macrolides (Pg-p)
The seven pillars of intracellular activity?

1. Penetration
2. No efflux
3. Accumulation

Much less obvious ...
no simple correlation accumulation-activity
ex.: fluoroquinolones, macrolides, β-lactams...
The seven pillars of intracellular activity?

1. Penetration
2. No efflux
3. Accumulation
4. Subcell. bioavailability

This is probably the most critical property
ex.: fluoroquinolones, oxazolidinones vs macrolides and aminoglycosides
The seven pillars of intracellular activity?

1. Penetration
2. No efflux
3. Accumulation
4. Subcell. bioavailability
5. Expression of activity

Interesting aspect but could vary for drugs and bugs ...
- one + example: intracellular MRSA and conventional β-lactams...
  (not shown in this lecture)
The seven pillars of intracellular activity?

1. Penetration
2. No efflux
3. Accumulation
4. Bacterial responsiveness (population)
5. Expression of activity
6. Bacterial responsiveness (population)
4. Subcell. bioavailability

Probably critical to explain the non-eradication or part of the intracellular inoculum…
→ future therapeutic targets?
The seven pillars of intracellular activity?

1. Penetration
2. No efflux
3. Accumulation
4. Subcell. bioavailability
5. Expression of activity
6. Bacterial responsiveness and pharmacodynamics
7. Cooper. with host def.

Not addressed here but probably very important
But what can we do NOW?

• All tested antibiotics fail to eradicate intracellular *S. aureus* (and many other bacteria) in the THP-1 model (and in other models)...

• Some antibiotics, however, fare better (moxifloxacin, oritavancin, e.g.) and could be our drugs of (desperate) choice...

• We must now try to understand the reasons for this global failure … and/or screen for better compounds *(follow us…)*

• In the meantime, intracellular organisms will remain a cause of concern and may (unfortunately) justify large doses and prolonged treatments… which is what we most often do…

15 June 2016 University of Notre Dame, IN
But this work would not have been possible without

The drugs…

- β-lactams: penicillin V, oxacillin, cloxacillin, ceftaroline*, ceftobiprole* (+ avibactam*)
- aminoglycosides: gentamicin, amikacin
- lincosamides: clindamycin, pirlimycin
- fluoroquinolones: ciprofloxacin, pefloxacin, lomefloxacin, sparfloracin, moxifloxacin, garenoxacin*, gemifloxacin, finafloxacin*, delafloxacin*
- oxazolidinones: linezolid, radezolid*, tedizolid*
- glycopeptides: vancomycin, telavancin*, oritavancin*,
- macrolides: clarithromycin, azithromycin, solithromycin*,
- other classes: daptomycin, GSK 1322322*, gepoditacin*, Debio1452*
- etc…

* new molecules studied at preclinical level

The people…

- M.B. Carlier *,**
- A. Zenebergh **
- B. Scorneaux *
- Y. Ouadrhiri *
- S. Caryn *,**
- C. Seral **
- M. Barcia-Macay *
- H.A. Nguyen **
- J.M. Michot *
- B. Marquez **
- C. Vallet *
- S. Lemaire *,**
- A. Melard
- J. Buyck **
- D. Das **
- F. Peyrusson *
- F. Van Bambeke (current head of the group)
- …

* doctoral fellow; ** post-doctoral fellow

Let us catch them