Intracellular Activity of Antibiotics: the knowns, the uncertainties and the failures

Paul M. Tulkens, MD, PhD *
Emeritus Professor of Pharmacology
Invited Lecturer
(Drug Discovery & Development / Rational) therapeutic choices)

Cellular and Molecular Pharmacology
Louvain Drug Research Institute
Health Science Sector
Université catholique de Louvain
Brussels, Belgium

PKUK 2015, Chester, UK, 18-20 November 2015
* with slides borrowed from Françoise Van Bambeke

With approval of the Belgian Common Ethical Health Platform – visa no. 15/V1/5450/073355
Disclosures and slides availability

• Research grants
  – Theravance, Astellas, Targanta, Cerexa/Forest, AstraZeneca, Bayer, GSK, Trius, Rib-X, Eumedica, Debiopharm
  – Belgian Science Foundation (F.R.S.-FNRS), Ministry of Health (SPF), Walloon and Brussels Regions, European Union (FP7 programme)

• Speaking fees
  – Bayer, GSK, Sanofi, Johnson & Johnson, OM-Pharma

• Decision-making and consultation bodies
  – 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
Chester extra and intra...
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
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 2 x
- ansamycins: 2-3 x
- tetracyclines: 2-4 x
- fluoroquinolones: 5 - 20 x
- macrolides: 4 to > 100 x *
- glycopeptides: 1 to 400 x !! **

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

1. diffusion

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

1. diffusion

Azithromycin accumulation in rat embryo fibroblasts

Tyteca et al., EJCB, 2001, in press
How do antibiotics penetrate in cells?

1. diffusion

Sparfloxacin accumulation in THP-1 macrophages

Ouadrhiri et al., AAC, 1999
How do antibiotics penetrate in cells?

1. diffusion

Ampicillin accumulation in THP-1 macrophages

Ouadrhiri et al., AAC, 1999
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
Carrier-mediated transport

OATPs, OATs and OCTs: the organic anion and cation transporters of the \textit{SLCO} and \textit{SLC22A} gene superfamilies

Megan Roth\textsuperscript{1}, Amanda Obaidat\textsuperscript{1} and Bruno Hagenbuch\textsuperscript{1,2}

\textsuperscript{1}Department of Pharmacology, Toxicology and Therapeutics, The University of Kansas Medical Center, Kansas City, KS, USA, and \textsuperscript{2}The University of Kansas Cancer Center, Kansas City, KS, USA

OATPs, OATs and OCTs: the organic anion and cation transporters of the SLCO and SLC22A gene superfamilies

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How do antibiotics penetrate in cells?

3. pinocytosis

- aminoglycosides
- glycopeptides
How do antibiotics penetrate in cells?

- aminoglycosides in fibroblasts

- Slow (days...)
- ill-effective (2-4 fold)

Cc/Ce = 1

Ce = 1.3 mg/ml
Ce = 0.65 mg/ml
Ce = 0.35 mg/ml

Tulkens & Trouet, 1978
How do antibiotics penetrate in cells?

Receptor-mediated pinocytosis in kidney cortex

Binding to:
- megalin (Moestrøp et al., 1995)
- acidic phospholipids (Humes et al., 1983)

Giuliano et al., J. Pharm. Exp. Ther., 1986
How do antibiotics penetrate in cells?

membrane binding and uptake of lipoglycopeptides

How do antibiotics penetrate in cells?

membrane binding and uptake of lipoglycopeptides

How do antibiotics penetrate in cells?

membrane binding and uptake of lipoglycopeptides

**TABLE 1. Oritavancin accumulation by different cell types**

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Accumulation ratio$^b$ (no. of determinations)</th>
</tr>
</thead>
<tbody>
<tr>
<td>J774 mouse macrophages</td>
<td>66.4 ± 11.8 (12)</td>
</tr>
<tr>
<td>THP-1 human monocytes</td>
<td>84.3 ± 7.0 (9)</td>
</tr>
<tr>
<td>Rat embryo fibroblasts</td>
<td>72.4 ± 9.4 (6)</td>
</tr>
<tr>
<td>LLC-PK1 pig kidney proximal tubular cells</td>
<td>37.8 ± 6.4 (3)</td>
</tr>
<tr>
<td>Caco-2 human colorectal cells</td>
<td>13.8 ± 0.4 (3)</td>
</tr>
</tbody>
</table>

$^a$ The cells were incubated for 2 h at 37°C with 25 mg of the drug per liter in a medium containing 10% FCS.

$^b$ Ratio of cellular concentration to extracellular concentration.

Efflux

http://www.tcdb.org/

Saier, 2000
Efflux

http://www.tcdb.org/

Transporter Classification Database

TCDB is operated by the Saier Lab Bioinformatics Group

- 1: Channels/Pores
- 2: Electrochemical Potential-driven Transporters
- 3: Primary Active Transporters
- 4: Group Translocators
- 5: Transmembrane Electron Carriers
- 8: Accessory Factors Involved in Transport
- 9: Incompletely Characterized Transport Systems

Saier, 2015
Some transporters involved in the efflux of antibiotics from eukaryotic cells

<table>
<thead>
<tr>
<th>superfamily</th>
<th>transporter</th>
<th>physiol. substrates</th>
<th>antibiotics</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABC</td>
<td>MDR1</td>
<td>phospholipids</td>
<td>fluoroquinolones, macrolides, β-lactams, tetracyclines, streptogramins</td>
</tr>
<tr>
<td></td>
<td>MRP1</td>
<td>phospholipids, leukotrienes, conjugates</td>
<td>fluoroquinolones, macrolides, rifamycins</td>
</tr>
<tr>
<td></td>
<td>MRP2 / 4</td>
<td>conjugates</td>
<td>fluoroquinolones, β-lactams</td>
</tr>
<tr>
<td>MFS</td>
<td>NPT1</td>
<td>phosphates</td>
<td>β-lactams</td>
</tr>
<tr>
<td>OAT</td>
<td>OATP1</td>
<td>bile salts, steroids</td>
<td>β-lactams</td>
</tr>
</tbody>
</table>
Examples of efflux-mediated control of cellular accumulation

1. fluoroquinolones

accumulation of ciprofloxacin in J774 macrophages

Evidencing active efflux ...

non linear accumulation kinetics ...

receptor mediated uptake

apparent facilitated uptake

diffusion

$Ce$

$Cc$
Evidencing active efflux ... 

non linear accumulation kinetics ... 

apparent facilitated uptake

by saturation of efflux!
Influence of efflux inhibitors on fluoroquinolones and macrolide accumulation...

TABLE 1. Influence of efflux pump modulators on the accumulation of ciprofloxacin and azithromycin by J774 macrophages

<table>
<thead>
<tr>
<th>Modulator</th>
<th>Concen</th>
<th>Increase in accumulation (% of controls)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Ciprofloxacin(^b)</td>
</tr>
<tr>
<td>Probenecid</td>
<td>2.5 mM</td>
<td>288 ± 4</td>
</tr>
<tr>
<td>Gemfibrozil</td>
<td>200 μM</td>
<td>308 ± 3</td>
</tr>
<tr>
<td>MK571</td>
<td>200 μM</td>
<td>392 ± 5</td>
</tr>
<tr>
<td>Verapamil</td>
<td>100 μM</td>
<td>136 ± 4</td>
</tr>
<tr>
<td>Cyclosporin A</td>
<td>50 μM</td>
<td>236 ± 5</td>
</tr>
<tr>
<td>GF 120918</td>
<td>1 μM</td>
<td>116 ± 2</td>
</tr>
</tbody>
</table>

\(^a\) Statistical analysis: for ciprofloxacin, all differences are significant; for azithromycin, differences are significant for verapamil, cyclosporin A, and GF 120918 only (paired \(t\) test compared to controls).

\(^b\) 17 mg/liter (50 μM); 2-h incubation.

\(^c\) 5 mg/liter (6.8 μM); 3-h incubation (time to equilibrium).

\(^d\) ND, not determined.

\(^e\) 20 μM.

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

- Cytosol:
  - Fluoroquinolones
  - Beta-lactams
  - Ansamycins
  - Macrolides (1/3)

- Endosomes

- Phagolysosomes:
  - Macrolides (2/3)
  - Aminoglycosides

- Phagosomes
Subcellular localization is often studied by cell fractionation techniques.
A recent example with two novel oxazolidinones: 1. tedizolid (accumulation)

Comparative accumulation of linezolid (LZD) and of tedizolid (TR-700) in THP-1 macrophages
(a) Uptake kinetics
(b) Influence of the temperature (2 h incubation)

Subcellular localization of the accumulated tedizolid … or redistribution?

Tedizolid subcellular distribution in extract from J774 macrophages

Mechanisms of localisation and accumulation in cytosol ...

- β-lactams
- fluoroquinolones
- non ionic oxazolidinones

Loose binding to cytosol soluble constituents?

OR

leakage from other sites?
Accumulation of radezolid

FIG. 2. Kinetics of radezolid uptake and release within THP-1 and J774 cell lines and PMNs. Left, uptake. Cells were incubated for up to 5 h in the presence of 4 mg/liter radezolid (RDZ) or 250 mg/liter linezolid (LZD). The ordinate shows the apparent cellular-to-extracellular concentration ratio. Data are fitted to one-phase exponential association ($R^2 = 0.837$ for RDZ, 0.824 for linezolid). Right, release. Cells were incubated with 4 mg/liter radezolid during 2 h before being transferred to drug-free medium. Values are expressed as the percentage of the accumulated amount at 2 h (time zero). Data are fitted to one-phase exponential decay ($R^2 = 0.988$). Results are given as means ± standard deviations ($n = 3$).
Subcellular localization of radezolid

<table>
<thead>
<tr>
<th>tracer</th>
<th>% of total amount in the cell extract</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S</td>
</tr>
<tr>
<td>Radezolid</td>
<td>58.2</td>
</tr>
<tr>
<td>NAB</td>
<td>10.6</td>
</tr>
<tr>
<td>CytOx</td>
<td>1.7</td>
</tr>
<tr>
<td>LDH</td>
<td>97.0</td>
</tr>
</tbody>
</table>

Mechanisms of localisation and accumulation ...

- proton trapping (ML, OZ)
- binding to phospholipids?
- for aminoglycosides: inability to cross membranes

- macrolides
- aminoglycosides
- cationic oxazolidinones
Mechanisms of localisation and accumulation ...

- Increase in phospholipid cellular content

- Potential toxicity?

Azithromycin extracellular concentration (mg/l) vs. cellular concentration (mg/l)

Van Bambeke et al., JAC, 1998
So, what we know in a nutshell ...

<table>
<thead>
<tr>
<th>Pharmacological class</th>
<th>Antibiotic</th>
<th>Accumulation level at equilibrium (C_d/C_e)(^a)</th>
<th>Cellular concentration at equilibrium (mg/l)(^b)</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</td>
<td>2/3 Lysosomes, 1/3 Cytosol</td>
</tr>
<tr>
<td></td>
<td>Clarithromycin, Roxithromycin, Telithromycin</td>
<td>10 to 50</td>
<td>~ 20 to 400</td>
<td>(a few hours)</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, Levofloxacin, Grepafloxacin</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>Moxifloxacin, Garenoxacin, Gemifloxacin</td>
<td>10 to 20</td>
<td>~ 40 to 80</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>Unknown</td>
<td>Unknown</td>
</tr>
</tbody>
</table>

But where does this lead us for activity?

* taken from a slide presented at ECCMID in 2002

Ph. Geluck, with permission
Intracellular activity of antibiotics

• What has been know for long about pharmacokinetics…

• What has surprised us …

• Adding pharmacodynamics …

• A renewed model ?
First Illustration: the Listeria Story

**antibiotics:**

- ampicillin
- azithromycin
- sparfloxacin

*Listeria monocytogenes hly+*
Intracellular infection cycle of *Listeria monocytogenes* hly⁺

from Portnoy et al.
Following the intracellular fate of *Listeria m.* by EM

- **A** phagocytosis
- **B** escape from vacuole
- **C** in cytosol
MIC, accumulation and activity against cytosolic *Listeria m.* ...

- **MIC**
  - AMPI: 0.0, 0.5, 1.0, 1.5
  - AZ: 0.0, 0.5, 1.0, 1.5
  - SP: 0.0, 0.5, 1.0, 1.5

- **Accumulation**
  - AMPI: 0, 25, 50, 75
  - AZ: 0, 25, 50, 75
  - SP: 0, 25, 50, 75

- **Activity** *
  - AMPI: 0, 0.5, 1.0
  - AZ: 0, 0.5, 1.0
  - SP: 0, 0.5, 1.0

* ∆ log CFU 5h Ce = 10 x MIC

Ouadhriri et al., AAC, 1999
To make a long story short: can we predict intracellular activity as a function of the accumulation of antimicrobial agents?


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
To make a long story short: can we predict intracellular activity as a function of the accumulation of drug concentrations?

**Diagram:**

- **Listeria monocytogenes**
  - Δ log CFU from time 0 vs. Log cellular concentration (concentration in mg/l)
  - Drugs: AMP, AZM, CIP, ETP, GEN, GRN, LVX, MXF

- **Staphylococcus aureus**
  - Δ log CFU from time 0 vs. Log cellular concentration (concentration in mg/l)
  - Drugs: AMP, AZM, CIP, ETP, GEN, GRN, LNZ, LVX, MXF, OXA, ORI, PEN V, RIF, TEC, TEL, VAN

**Legend:**

- 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

To make a long story short: can we predict intracellular activity as a function of the accumulation.

**Diagram:**

- **Listeria monocytogenes**
  - MXF
  - GRN
  - LVX
  - MEM
  - AMP
  - AZM
  - ETP
  - GEN

- **Staphylococcus aureus**
  - MXF
  - GRN
  - LVX
  - OXA
  - AMP
  - NAF
  - RIF
  - VAN
  - TEC
  - TEL
  - PEN V
  - LNZ
  - GEN

**Key:**
- 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

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.
Subcellular bioavailability of antibiotics?

aminoglycosides, poorly diffusible drugs (oritavancin, e.g.) or subjected to proton-trapping sequestration (macrolides, e.g.) may remained confined therein ...
Intracellular activity of antibiotics

• What has been know for long about pharmacokinetics…

• What has surprised us …

• Adding pharmacodynamics …

• A renewed model ?
Second illustration: the 24h 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)

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

Typical post-phagocytosis inoculum:
- 5 to 7x10⁵ CFU/mg prot.

• Cell washing, collection, and lysis
• Cell-associated CFUs counting
• Cell Protein content determination

This example is for S. aureus. Similar design for other bacteria
2. Analysis of the response

$E_{\text{min}}$: cfu increase (in log$_{10}$ units) at 24 h from the corresponding initial inoculum as extrapolated for an infinitely low antibiotic concentration.

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

$E_{\text{max}}$: cfu decrease (in log$_{10}$ units) at 24 h from the corresponding initial inoculum as extrapolated from infinitely large antibiotic concentration.

Second illustration: the 24h dose-effect model

2. the analysis of the response

\[ E_{\text{min}}: \text{cfu increase (in log}_{10}\text{ units) at 24 h from the corresponding initial inoculum as extrapolated for an infinitely low antibiotic concentration} \]

\[ \Delta \text{Log}_{10} \text{of extracellular concentration (x MIC)} \]

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

\[ E_{\text{max}}: \text{cfu decrease (in log}_{10}\text{ units) at 24 h from the corresponding initial concentration} \]

Question #1: does increased accumulation of a given antibiotic results in its increased potency and maximal activity?

Question #2: does difference in accumulation of antibiotics of the same class results in commensurate differences in potency and maximal activity?
Question #2: does difference in accumulation of antibiotics of the same class results in commensurate differences in potency and maximal activity?

1. accumulation (Ce = 20 mg/L)
Question #2: does difference in accumulation of antibiotics of the same class results in commensurate differences in potency and maximal activity?

1. accumulation (Ce = 20 mg/L)

2. activity (MIC = 0.5 – 2 mg/L)

no difference in dose-effect relationship!
Question #2: does difference in accumulation of antibiotics of the same class results in commensurate differences in potency and maximal activity?

1. accumulation (Ce = 20 mg/L)

3. intracellular concentration to obtain a given effect

You need more of the drug that accumulates more.
Question #3: are antibiotics that accumulate more effective (potency and maximal activity) than those which do not?


<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Cmax (X MIC)</th>
<th>log CFU (24 h - 0 h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxacillin</td>
<td>-2, -1, 0</td>
<td>intra: 2.5, extra: 0.0</td>
</tr>
<tr>
<td>Moxifloxacin</td>
<td>-2, -1, 0</td>
<td>intra: 2.5, extra: 0.0</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>-2, -1, 0</td>
<td>intra: 2.5, extra: 0.0</td>
</tr>
<tr>
<td>Teitavancin</td>
<td>-2, -1, 0</td>
<td>intra: 2.5, extra: 0.0</td>
</tr>
</tbody>
</table>

The answer is not obvious!
Question #4: why are antibiotics unable **eradicate** the intracellular bacteria (viz. low maximal efficacy)?

\[ S. \text{ aureus} \text{ model (ATCC25223)} \]


![Graph comparing extracellular and intracellular bacterial growth](chart.png)

**compare the extracellular and the intracellular** E\(_{\text{max}}\)
Question #4: why are antibiotics unable **eradicate** the intracellular bacteria (viz. low maximal efficacy)?

**S. aureus model** (ATCC33591 [MRSA])

about question #4 (eradication): some do (slightly) better than others (viz. **maximal** efficacy)?
about question #4 (eradication): some do (slightly) better than others (viz. maximal efficacy) but all do less than in broth ...
about question #4 (eradication): some do (slightly) better than others (viz. maximal efficacy) but all do less than in broth ...

A more systematic comparison with ATCC 25983 (S. aureus) and at human C\textsubscript{max}

From high to low intracellular activity:

- ORI = oritvancin
- MXF = moxifloxacin
- GRN = garenoxacin
- LVX = levofloxacin
- CIP = ciprofloxacin
- AMP = ampicillin
- RIF = rifampincin
- TEC = teicoplann
- NAF = nafcillin
- VAN = vancomycin
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• What has surprised us …

• Adding pharmacodynamics …

• A renewed model ?
The seven pillars of intracellular activity?

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 (Pgp)
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. Subcell. bioavailability
5. Expression of activity
6. Bacterial responsiveness (population)

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
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.
So, it a nutshell…

from ancient

to contemporary

but still a lot of unknowns…
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, sparfloxacin, 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…

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* 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)
• …

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* doctoral fellow; ** post-doctoral fellow