Study of intracellular activity of antibiotics: significance for the clinical practice

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* with slides borrowed from Françoise Van Bambeke and Frédéric Peyrusson
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.facman.ucl.ac.be → Lectures
you often need to go deep to be surprised…
Why do we wish to look at intracellular activity of antibiotics?

• Beyond truly obligate intracellular parasites (e.g., *Legionella*, *Chlamydia*, *Mycobacteriae*, …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 *S. aureus*

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

Van Bambeke & Tulkens unpublished

**S. aureus** in human osteoblasts


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

Horst *et al.* Am J Pathol 2012;181:1206–1214 - PMID: 22902429
Are antibiotics active at all in cells? 1

Control (no antibiotic) 2

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

Oritavancin 25 mg/L
(= \(C_{\text{max}}\) 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 know for long about pharmacokinetics…

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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: \( \leq 1x \)
- 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,
- ...

26 May 2016

XVIII международный конгресс по антимикробной терапии
How do antibiotics penetrate in cells?

2. carrier-mediated influx

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

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

3. Pinocytosis

- Aminoglycosides
- Glycopeptides
How do antibiotics penetrate in cells?

- 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: 7097555

- Receptor-mediated pinocytosis in kidney cortex
  - Giuliani et al. J Pharmacol Exp Ther 1986;236:470-5 - PMID: 3944768
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)

- lysosomosomes
  - macrolides (2/3)
  - aminoglycosides

- endosomes
- phagosomes
- phagolysosomes

??
### So, what we know in a nutshell ...

<table>
<thead>
<tr>
<th>Pharmacological class</th>
<th>Antibiotic</th>
<th>Accumulation level at equilibrium ($C_d/C_e$)</th>
<th>Cellular concentration at equilibrium (mg/l)</th>
<th>Time to equilibrium</th>
<th>Predominant subcellular localization</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>β-Lactams</strong></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>
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<tr>
<td></td>
<td>Grepafloxacin</td>
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</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>
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</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></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>
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<tr>
<td>Ansamycins (rifamycins)</td>
<td>Rifampin</td>
<td>2 to 10</td>
<td>~ 36 to 180</td>
<td>Unknown</td>
<td>Unknown</td>
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<tr>
<td></td>
<td>Rifapentine</td>
<td>60 to 80</td>
<td>~ 1200 to 1600</td>
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<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>
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<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?
Intracellular activity of antibiotics

• What has been know for long about pharmacokinetics …

• What has surprised us …

• Adding pharmacodynamics …

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

\[ \log \text{CFU from time 0} \]

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

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

**Abbreviations**
- 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

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Intracellular activity is not directly correlated to accumulation

\[ \text{Log cellular concentration (concentration in mg/l)} \]

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

**Staphylococcus aureus**
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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?

<table>
<thead>
<tr>
<th>High</th>
<th>Fair</th>
<th>Nil</th>
</tr>
</thead>
<tbody>
<tr>
<td>FQ / oxazolidinones / β-lactams</td>
<td>ML / AG</td>
<td></td>
</tr>
</tbody>
</table>
Subcellular bioavailability of antibiotics?

Fluoroquinolones, β-lactams, oxazolidinones, ... may move easily across membranes.
Subcellular bioavailability of antibiotics?

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 known for long about pharmacokinetics…

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• 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

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

**Phagocytosis** (1 h)

**Extracellular Wash**
GEN 50 µg/mL
(45 min)

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

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

2. Analysis of the response

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

\[ \log_{10} \text{of extracellular concentration (× MIC)} \]

**\( E_{\text{min}} \):** 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

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

2. the analysis of the response

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

Log$_{10}$ of extracellular concentration (× MIC)

$\Delta$ Log$_{10}$ cfu (24 h – 0 h)

Are intracellular and extracellular activities equal?

S. aureus model (ATCC25223)


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

**S. aureus model**

(ATCC33591 [MRSA])

![Graph showing concentration-dependent activities of four antibiotics](image)

- 5 log !
- 1 log !

**Figure 1.** Concentration-dependent activities of four antibiotic agents against extracellular (IMHB broth pH 7.4 (a)) and intracellular (THP-1 monocytes (b)) forms of S. aureus strain ATCC 33591 (MRSA). For these experiments, broths or infective cells were incubated at $37^\circ\text{C}$ in the presence of increasing concentrations of antibiotic (total drug; abscissa). The ordinates show the change in the log of the colony-forming units per ml of 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 mean + SD bars, smaller than the size of the symbols. The lowest limit of detection corresponds to a cfu equal to the original inoculum. The grey zone shows the range of maximal serum concentrations observed in healthy patients on the following reported $C_{\text{max}}$ values: ceftaroline, 21 mg/L; vancomycin, 20–50 mg/L; daptomycin, 5–10 mg/L. (footnote c in Table 2).

### A comparison of $E_{\text{max}}$...  

<table>
<thead>
<tr>
<th>Antibiotic Class</th>
<th>Molecule *</th>
<th>Extracellular **</th>
<th>intracellular</th>
</tr>
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<tbody>
<tr>
<td><strong>beta-lactams</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>oxacillin 1</td>
<td>-3.1</td>
<td>-1.6</td>
<td></td>
</tr>
<tr>
<td>ceftaroline 2</td>
<td>-5.4</td>
<td>-0.6</td>
<td></td>
</tr>
<tr>
<td><strong>lipopeptides</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>daptomycin 2</td>
<td>-5.1</td>
<td>-1.0</td>
<td></td>
</tr>
<tr>
<td><strong>fluoroquinolones</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>moxifloxacin 4</td>
<td>-4.8</td>
<td>-2.0</td>
<td></td>
</tr>
<tr>
<td>ciprofloxacin 5</td>
<td>-4.9</td>
<td>-1.6</td>
<td></td>
</tr>
<tr>
<td><strong>pyrrolocytosines</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RX-P873 6</td>
<td>-4.2</td>
<td>-0.7</td>
<td></td>
</tr>
<tr>
<td><strong>peptides (defensins)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NZ2114 7</td>
<td>-4.1</td>
<td>-1.5</td>
<td></td>
</tr>
<tr>
<td><strong>deformylase inhibitors</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GSK1322322 3</td>
<td>-4.8</td>
<td>-0.4</td>
<td></td>
</tr>
<tr>
<td><strong>glycopeptides</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
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<td>vancomycin 2</td>
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<td>-0.6</td>
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<tr>
<td><strong>lipoglycopeptides</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>oritavancin 1</td>
<td>-5.5</td>
<td>-3.1</td>
<td></td>
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<tr>
<td><strong>oxazolidinones</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<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  

References:  
1. AAC (2006) 50:841-851;  
4. JAC (2011) 66:596-607;  
5. IJAA (2011) 38:52-59;  
7. JAC (2010) 65:1720-1724

*a Reminder: $E_{\text{max}}$ is the maximal reduction of the initial inoculum for an infinitely large drug concentration*
# A comparison of $E_{\text{max}}$... a

<table>
<thead>
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<tr>
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<td>moxifloxacin</td>
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<td>NZ2114</td>
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<td>-1.5</td>
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<tr>
<td><strong>deformylase inhibitors</strong></td>
<td>GSK233232</td>
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<td>-0.4</td>
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<tr>
<td><strong>glycopeptides</strong></td>
<td>vancomycin 2</td>
<td>-5.1</td>
<td>-0.6</td>
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<tr>
<td><strong>lipoglycopeptides</strong></td>
<td>oritavancin 1</td>
<td>-5.5</td>
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<td><strong>oxazolidinones</strong></td>
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a Reminder: $E_{\text{max}}$ is the maximal reduction of the initial inoculum for an infinitely large drug concentration

---

*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 ((\Delta \log_{10} CFU) at 24h)</th>
<th>Extracellular **</th>
<th>intracellular</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<tr>
<td>beta-lactams</td>
<td>oxacillin (^1)</td>
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</tr>
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<td></td>
<td>ceftaroline (^2)</td>
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<td></td>
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* all molecules but linezolid are highly bactericidal by conventional MBC/MIC measurements
** limit of detection: -5.5 log10 units

References:

\(^a\) Reminder: \(E_{\text{max}}\) 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 (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)

5. Expression of activity
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
So, what is the clinical significance?

- 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…
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…

* new molecules studied at preclinical level

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