Pharmacological approaches to the discovery and optimized development of novel antibiotics

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Louvain Drug Research Institute
Université catholique de Louvain
Brussels, Belgium
The approach in a nutshell

antibiotics: from molecules to man

pharmacodynamics  
pharmacokinetics  
antibiotic toxicity  
clinical applications  
resistance  
novel bacterial targets
What will it be all about?

• The antibiotic crisis …
  – are antibiotics following a **path of madness**?
    (the reality in hospitals and in the community…)
  – the "**resistome**" (or why do we will always have resistance…)
  – the “**selectome**” (or why do we favor emergence of resistance)
  – the “**connectome**” (or why we loose several antibiotics at the same time)

• The main lines of action (for research)
  ➢ the 7 pillars of wisdom?

• Laboratory and translational studies at LDRI (examples)
  – poorly exploited targets (D-Ala-D-Ala ligase)
  – refurbishing old antibiotics (aminogycosides, polymyxins, temocillin)
  – better antibiotic use (PK/PD, intracellular bacteria)
  – PK/PD approaches to mitigate the emergence of resistance (β-lactams and fluoroquinolones)
Are antibiotics following a path to madness?

Discovery in soil bacteria and fungi

1928 - …
Are antibiotics following a path to madness?

and then we all saw the blooming tree of semi-synthetic and totally synthetic antibiotics

1950 – 1980 …
Are antibiotics following a path to madness?

and the US General Surgeon told us that the fight was over 1970 …
Are antibiotics following a path to madness?

But...
Resistance of *P. aeruginosa* in hospitals
(International data – EUCAST breakpoints)

![Graphs showing resistance levels of *P. aeruginosa* to various antibiotics over time.](image.png)

Spreading of NDM-1 in the community …

Outbreak of Carbapenem-Resistant Enterobacteriaceae Containing \( \text{bla}_{\text{NDM-1}} \)
Ontario, Canada

Sergio Borgia,1,2,8 Olga Lastovetska,4,5 David Richardson,1,2,8 Alireza Eshaghi,4 Jianhui Xiong,3 Catherine Chung,6 Mahin Baqi,1,2 Allison McGeer,5,6,7 Gloria Ricci,2 Rachael Sawicki,3 Rajni Pantelidis,3 Donald E. Low,4,5,6 Samir N. Patel,5,6 and Roberto G. Melano1,5,6,8

1Division of Infectious Diseases, 2Department of Laboratory Medicine, and 3Infection Prevention and Control, William Osler Health System, Brampton, 4Public Health Ontario, Public Health Laboratories, 5Department of Laboratory Medicine and Pathobiology, University of Toronto, 6Department of Microbiology and 7Infection Prevention and Control, Mount Sinai Hospital, Toronto, and Departments of 8Medicine and 9Pathology and Molecular Medicine, McMaster University, Hamilton, Ontario, Canada


NDM-1-Producing \( \text{Klebsiella pneumoniae} \) Resistant to Colistin in a French Community Patient without History of Foreign Travel

Corinne Arpin,6 Patrick Noury,6 Delphine Boraud,6 Laure Coulangé,6 Alain Manetti,6 Catherine André,6 Fatima M’Zali,6 and Claudine Quentin6

Université de Bordeaux, Microbiologie Fondamentale et Pathogénicité UMR 5234, Bordeaux, France; Laboratoire de Biologie Médicale EXALAB, Site de Villenave-d’Ornon, Villenave d’Ornon, France; and Agence Régionale de Santé, Espace Rodesse, Bordeaux, France

A carbapenem-resistant \( \text{Klebsiella pneumoniae} \) strain, Kp5196, was responsible for an uncomplicated cystitis in a patient living at home and without history of foreign travel. This isolate produced the metallocarbapenemase NDM-1 and was resistant to all antibiotics except tetracyclines and colistin. The \( \text{K. pneumoniae} \) strain belonged to sequence type ST15, and \( \text{bla}_{\text{NDM-1}} \) was carried by a nontypeable conjugative plasmid. Two months later, a similar ST15 isolate, Kp5241, was present in the patient but was additionally colistin resistant.

The resistome …

• Resistance emergence is a natural process that has gone on for time immemorial.
  - Example: Parts of the operon mediating *vancomycin resistance* have been found in the permafrost layer, demonstrating the ancient nature of the problem…
    (many other examples of “resistance” in pre-antibiotic era)
  - Significance: resistance was with us since ever and we will never get rid of it …

• Horizontal gene transfer has long been considered as the main mechanism by which the resistome has been built over years
  - β-actamases, MRSA (PBP2a), Penicillin-resistant *S. pneumoniae* (mosaic genes), aminoglycoside-inactivating enzymes, QnR (fluoroquinolones-target protecting protein) …
The resistome …

The antibiotic resistome.

• all the genes and their products that contribute to antibiotic resistance.

• highly redundant and interlocked system

• clinical resistance under represents the resistance capacity of bacteria.

• existing biochemical mechanisms (protoresistome) serve as a deep reservoir of precursors that can be co-opted and evolved to

http://www.nap.edu/openbook.php?record_id=12925
Clinical resistance: the tip of the iceberg?

• **“Clinical” resistance genes** are found on **pathogenic bacteria.** These are the fewest but also the most problematic ones at present.

• **“Father resistance genes”** found on antibiotic producers. (microorganisms that naturally produce antibiotics have their own protection mechanisms to avoid the adverse effects of the antibiotics on themselves).

  ➢ **These genes are a strong source for the pathogenic bacteria.**

• **Cryptic resistance genes.**
  (genes are embedded in the bacterial chromosome that may be overexpressed when “needed”)

• **Precursor genes.**
  (encode proteins with basal level activity against antibiotics but may evolve to a “full resistance genes” given the appropriate selection pressure.)
“Father resistance genes”: an original example with aminoglycosides

Proc. Nat. Acad. Sci. USA
Vol. 70, No. 8, pp. 2276–2280, August 1973

Aminoglycoside Antibiotic-Inactivating Enzymes in Actinomycetes Similar to Those Present in Clinical Isolates of Antibiotic-Resistant Bacteria

(streptomycyes/origin of R-factors/gentamicin-acetate)

RAOUL BENVENISTE* AND JULIAN DAVIES†
Department of Biochemistry, College of Agricultural and Life Sciences, University of Wisconsin—Madison, Madison, Wis. 53706
Communicated by Henry Lardy, May 11, 1973

One of the most striking properties of the actinomycetes is the extent to which they produce antibiotics; most of the aminoglycoside antibiotics (streptomycin, neomycin, kanamycin, gentamicin, tobramycin, and lividomycin) are produced by them.
The selectome

A simple application of Darwin’s principles ...

selection pressure

 genes

 enzymes / nucleoproteins

 function

Detail of watercolor by George Richmond, 1840. Darwin Museum at Down House
How and why can you select so easily?

A simple application of Darwin’s principle…
to a highly plastic material…

- an infectious focus typically contains more than $10^6 - 10^9$ organisms

- most bacteria multiply VERY quickly (20 min…) and do mistake …

- they are not innocent or useless mistakes

**fast selection of the fitest!**
The hidden risk of therapy (in our hospitals ...)

In vivo development of antimicrobial resistance in *Pseudomonas aeruginosa* strains isolated from the lower respiratory tract of Intensive Care Unit patients with nosocomial pneumonia and receiving antipseudomonal therapy

Mickaël Riou\(^a\), Sylviane Carbonnelle\(^a\), Laëtitia Avrain\(^a\), Narcisa Mesaros\(^a\), Jean-Paul Pirnay\(^c\), Florence Bilocq\(^c\), Daniel De Vos\(^c,d\), Anne Simon\(^e\), Denis Piérard\(^f\), Frédérique Jacobs\(^g\), Anne Dediste\(^h\), Paul M. Tulkens\(^a,e\), Françoise Van Bambek\(^a\), Youri Glupczynski\(^i\)

\(^a\) Unité de Pharmacologie Cellulaire et Moléculaire & Louvain Drug Research Institute, Université catholique de Louvain, Brussels, Belgium

\(^b\) Coris BioConcept, Gembloux, Belgium

\(^c\) Laboratory for Molecular & Cellular Technology, Queen Astrid Military Hospital, Neder-over-Heembeek, Brussels, Belgium

\(^d\) Department of Molecular and Cellular Interactions, Vrije Universiteit Brussel, Brussels, Belgium

\(^e\) Laboratoire de Microbiologie, Cliniques Universitaires St-Luc, Brussels, Belgium

\(^f\) Laboratorium voor Microbiologie, Universitair Ziekenhuis Brussel, Brussels, Belgium

\(^g\) Clinique des Maladies Infectieuses, Hôpital Erasme, Brussels, Belgium

\(^h\) Laboratoire de Microbiologie, Centre Hospitalier Universitaire Saint-Pierre, Brussels, Belgium

\(^i\) Laboratoire de Microbiologie, Cliniques Universitaires UCL de Mont-Godinne, Yvoir, Belgium
Do you remain effective while treating?

- D0: initial isolate
- DL: last isolate obtained
- Individual values with geometric mean (95% CI)
- S (lowest line) and R (highest line) EUCAST breakpoints

* p < 0.05 by paired t-test (two-tailed) and Wilcoxon non-parametric test

a p < 0.05 by Wilcoxon non-parametric test only

Note: stratification by time between D0 and DL gave no clue (too low numbers)

Message: for all antibiotics, we see global increases of MIC during treatment
Actually, selecting for resistance is easy even in a closed system...

Exposure of *E. aerogenes* to anti-Gram (-) β-lactams to 0.25 MIC for 14 days with daily readjustment of the concentration based on MIC determination

<table>
<thead>
<tr>
<th>strains</th>
<th>Initial MIC (mg/L) a</th>
<th>TEM-exposed MIC (mg/L)</th>
<th>Revertant MIC (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TEM</td>
<td>FEP</td>
<td>MEM</td>
</tr>
<tr>
<td>2114/2 c</td>
<td>8</td>
<td>2</td>
<td>0.25</td>
</tr>
<tr>
<td>2502/4 c</td>
<td>8</td>
<td>2</td>
<td>0.125</td>
</tr>
<tr>
<td>3511/1 c</td>
<td>32</td>
<td>2</td>
<td>0.125</td>
</tr>
<tr>
<td>7102/10 d</td>
<td>512</td>
<td>32</td>
<td>1</td>
</tr>
</tbody>
</table>

a figures in bold indicate values > the R breakpoint for Enterobacteriaceae (EUCAST for MEM [8] and FEP [4]; BSAC and Belgium for TEM [16])
b dotblot applied with antiOmp36 antibody; signal quantified for grey value after subtraction of the signal of a porin-negative strain (ImageJ software); negative values indicate a signal lower than the background
c ESBL TEM 24 (+); d ESBL (-) and AmpC (+) [high level]; e Intermediate (I) according to EUCAST

Nguyen *et al.* (post-doc at LDRI) presented at the 8th ISAAR, Seoul, Korea, 8 April 2011 and additional work in progress
A simple experiment …

Exposure of *E. aerogenes* to anti-Gram (-) β-lactams to 0.25 MIC for 14 days with daily readjustment of the concentration based on MIC determination

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<td>2</td>
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* ESBL TEM 24 (+) ; d ESBL (-) and AmpC (+) [high level] ; e Intermediate (I) according to EUCAST

The connectome....
(cross-resistance)

http://wrightlab.mcmasteriiddr.ca/
Potential lines of action

ESSAY

Tackling antibiotic resistance


Nature Reviews Microbiology 9, 894-896 (December 2011)
7 pillars of wisdom?

1. Public education
2. Public health, sanitation and quality of life
3. New antibiotics → new / poorly exploited targets
4. Old antibiotics
5. Better antibiotic use
6. Alternatives to antibiotics
7. Collaborative approach

Bush et al. Nature Reviews Microbiology 9, 894-896 (December 2011)
Poorly exploited targets: D-Ala-D-Ala ligase

**D-Ala-D-X ligases**
- act in the very early steps of peptidoglycan synthesis
- are essential enzymes for bacterial growth

![Diagram showing the synthesis of peptidoglycan](image-url)
Rationale for a valid target …

- D-Ala-D-Ala ligases are essential enzymes
- This target has been only poorly explored
  - cycloserine: poor inhibitor and toxic)
  - Phosphinates: active on the enzyme but do not penetrate in the bacteria (too polar)
- Two approaches:
  - through conventional pharmacochemical approaches (modeling around know substrate)
  - de novo modeling from analysis of the protein conformation
  - BUT always using compounds that will enter the bacteria
Benzoxazoles

CLAIMS

1. A compound of any of formulas (I-a), (II-a), (III-a), (IV-a) or (V-a):

![Chemical structures](image)

or a pharmaceutically acceptable N-oxide form, addition salt, prodrug or solvate thereof, for use in the treatment of a bacterial infection.
Other molecules...

<table>
<thead>
<tr>
<th>Molecule Type</th>
<th>Structure</th>
<th>Number of Molecules</th>
</tr>
</thead>
<tbody>
<tr>
<td>Semicarbazides</td>
<td><img src="image" alt="Semicarbazides Structure" /></td>
<td>17 molecules</td>
</tr>
<tr>
<td>Triazoles</td>
<td><img src="image" alt="Triazoles Structure" /></td>
<td>8 moléules</td>
</tr>
<tr>
<td>Dioxo-Indolines</td>
<td><img src="image" alt="Dioxo-Indolines Structure" /></td>
<td>7 moléules</td>
</tr>
</tbody>
</table>
Semi-carbazides are better ...
7 pillars of wisdom....

1. Public education
2. Public health, sanitation and quality of life
3. New antibiotics → new / poorly exploited targets
4. Old antibiotics
   → aminoglycosides – polymyxins - temocillin
5. Better antibiotic use
6. Alternatives to antibiotics
7. Collaborative approach
Novel aminoglycosides *

• Advantages
  – wide spectrum and highly bactericidal
  – no metabolism and linear pharmacokinetics
  – extensive knowledge of their therapeutic and toxicological properties (leading to simple "once-daily dosing")

• Challenges
  – extensive development of resistance (mostly enzyme-mediated → aminoglycoside-modifying enzymes [AME])
  – nephrotoxicity and ototoxicity remain of concern and seem linked to activity

* using proprietary data of Achaogen Inc., South San Francisco, Cal. and example of collaborative approach
Aminoglycosides: starting from academic expertise in resistance

MINIREVIEW

Aminoglycosides: Activity and Resistance

MARIE-PAULE MINGEOT-LECLERCQ,¹* YOURI GLUPCZYNISKI,² AND PAUL M. TULKENS¹

Unité de Pharmacologie Cellulaire et Moléculaire, Université Catholique de Louvain, Brussels,¹ and Service de Microbiologie, Cliniques Universitaires UCL de Mont-Godinne, Yvoir,² Belgium
Main aminoglycoside-degrading enzymes...

FIG. 3. Major aminoglycoside-modifying enzymes acting on kanamycin B (this aminoglycoside is susceptible to the largest number of enzymes). Each group of enzymes inactivates specific sites, but each of these sites can be acted upon by distinct isoenzymes (roman numerals) with different substrate specificities (phenotypic classification; each phenotype comprises several distinct gene products [denoted by lowercase letters after the roman numeral in the text]); at least one enzyme is bifunctional and affects both positions 2' (O-phosphorylation) and 6' (N-acetylation). The main clinically used aminoglycosides on which these enzymes act are as follows: amikacin (A), dibekacin (Dbk), commercial gentamicin (G) (see text), gentamicin B (Gmb), kanamycin A (K), isepamicin (I), netilmicin (N), sisomicin (S), and tobramycin (T) (see text for discussion of arbekacin, sagamicin, and dactimicin). The drug abbreviations which appear in parentheses are those for which resistance was detectable in vitro even though clinical resistance was not conferred. Based on the data of Shaw et al. (89).
Academic expertise in nephrotoxicity

Aminoglycosides: Nephrotoxicity

MARIE-PAULE MINGEOT-LECLERCO* AND PAUL M. TULKENS

Unité de Pharmacologie Cellulaire et Moléculaire, Université Catholique de Louvain, Brussels, Belgium
FIG. 1. Ultrastructural alterations induced in proximal tubular cells during aminoglycoside treatment. (A) Control. Changes detected early on and at low doses (B) consist mainly of the enlargement of lysosomes, which most likely occurs by fusion of preexisting structures and which is caused by the progressive deposition of polar lipids which adopt a concentric lamellar disposition (myelin-like structures, most commonly referred to as myeloid bodies); the other subcellular structures are usually well preserved. Later changes or changes observed with high doses (C) include the apparent rupture of lysosomes (with the release of myeloid bodies in the cytosol), extensive mitochondrial swelling and damage, dilatation of the endoplasmic reticulum cisternae, shedding of the apical brush-border villi, pericytoplasmic membrane discontinuities, and the occurrence of apoptotic nuclei. These alterations do not necessarily coexist in all cells. The figure is adapted from reference 76 and is based on the typical descriptions given in references 38, 40, 71, 76, 77, 127, and 138.
Synthesis and Structure of the novel aminoglycoside ACHN-490

- ACHN-490 is a derivative of sisomycin (known to be highly active but toxic)
- The modifications made provide protection against most prevalent AMEs
- Equally active against gentamicin-S and gentamicin Enterobacteriaceae and Staphylococci
- Less toxic than gentamicin in *in vitro* and animal studies
- Indications currently tested include cUTI, HAP, cIAI, and blood stream infections

Aggen J, et al, ICAAC 2009 Poster F1-840
Activity of ACHN-490 against Contemporary Gram-Negative Clinical Isolates from Brooklyn, NY Hospitals

<table>
<thead>
<tr>
<th>Organism</th>
<th>Agent</th>
<th>MIC&lt;sub&gt;50&lt;/sub&gt;</th>
<th>MIC&lt;sub&gt;90&lt;/sub&gt;</th>
<th>%S</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>K. pneumoniae</em> (n=71)</td>
<td>ACHN-490</td>
<td>0.5</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Amikacin</td>
<td>16</td>
<td>64</td>
<td>58%</td>
</tr>
<tr>
<td></td>
<td>Gentamicin</td>
<td>1</td>
<td>&gt;64</td>
<td>59%</td>
</tr>
<tr>
<td></td>
<td>Imipenem</td>
<td>0.25</td>
<td>&gt;8</td>
<td>79%</td>
</tr>
<tr>
<td></td>
<td>Ceftazidime</td>
<td>&gt;16</td>
<td>&gt;16</td>
<td>37%</td>
</tr>
<tr>
<td></td>
<td>Ciprofloxacin</td>
<td>8</td>
<td>&gt;8</td>
<td>47%</td>
</tr>
<tr>
<td><em>E. coli</em> (n=32)</td>
<td>ACHN-490</td>
<td>1</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Amikacin</td>
<td>4</td>
<td>16</td>
<td>91%</td>
</tr>
<tr>
<td></td>
<td>Gentamicin</td>
<td>1</td>
<td>64</td>
<td>72%</td>
</tr>
<tr>
<td></td>
<td>Imipenem</td>
<td>0.12</td>
<td>8</td>
<td>82%</td>
</tr>
<tr>
<td></td>
<td>Ceftazidime</td>
<td>1</td>
<td>4</td>
<td>69%</td>
</tr>
<tr>
<td></td>
<td>Ciprofloxacin</td>
<td>&gt;8</td>
<td>&gt;8</td>
<td>31%</td>
</tr>
<tr>
<td><em>Enterobacter</em> spp. (n=30)</td>
<td>ACHN-490</td>
<td>1</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Amikacin</td>
<td>4</td>
<td>16</td>
<td>94%</td>
</tr>
<tr>
<td></td>
<td>Gentamicin</td>
<td>1</td>
<td>4</td>
<td>70%</td>
</tr>
<tr>
<td></td>
<td>Imipenem</td>
<td>0.5</td>
<td>2</td>
<td>94%</td>
</tr>
<tr>
<td></td>
<td>Ceftazidime</td>
<td>&gt;16</td>
<td>&gt;16</td>
<td>27%</td>
</tr>
<tr>
<td></td>
<td>Ciprofloxacin</td>
<td>0.12</td>
<td>1</td>
<td>74%</td>
</tr>
</tbody>
</table>

but the weakness is *Pseudomonas* (efflux)

Landman D, et al, ICAAC 2009 Poster F1-842
Extensive Safety Monitoring
Focused on Nephrotoxicity and Ototoxicity showed no major effect

- Adverse Event monitoring
- Routine safety laboratory assessments
- Renal
  - Daily BUN & Cr during dosing
  - Calculated Creatinine clearance using Cockroft-Gault formula
  - Measured Creatinine clearance based on 24-hour urine collection
  - Additional GFR monitoring through lothalamate clearance
- Cochlear
  - Full Audiograms with bone conduction
    - Test range 2 to 20 kHz (normal hearing range 2 to 8 kHz)
  - Daily Otoacoustic Emission (OAE) testing during multiple dose period
- Vestibular
  - Full Electronystagmography (ENG) with calorics
    - Tests: Unilateral Weakness, Directional Preponderance, Pendulum Tracking, Fixation
  - Daily Dynamic Visual Acuity (DVA) tests during multiple dose period
Why are aminoglycosides nephrotoxic?

1. Binding to brush border
2. Accumulation in lysosomes
Observation: aminoglycoside toxicity is **not** linked to peak ...

![Graph showing serum concentration of creatinine in rats after administration of gentamicin/kg per day in one, two, or three doses for two and 10 days.](image)

**Serum concentration of creatinine (mean ± SE) in rats after administration of 40 mg of gentamicin/kg per day in one, two, or three doses for two and 10 days.**

- **Daily dose divided in:**
  - Three doses/day
  - Two doses/day
  - One dose/day
  - Serum Creatinine
    - Mean ± 2 SE for 77 Control Rats

*From Bennett et al., J. Infect. Dis., 1979*
Aminoglycoside accumulation is kidney is saturable at clinically meaningful concentrations * ...

* Giuliano et al., J. Pharm. Exp. Ther., 1986

this is where patients are in a q8h schedule !!
Aminoglycoside peak / MIC ratio is predictive of clinical efficacy

Relationship between the maximal peak level/MIC ratio and the rate of clinical response. Vertical bars represent SE values.

Response rate (%) versus Maximum peak/mic ratio

ACHN-490: No Evidence of Nephrotoxicity Based on Daily Serum Creatinine

Bars = Min and Max
ACHN-490: No Evidence of Nephrotoxicity Based on Daily BUN Measurements

Bars = Min and Max
ACHN-490: No Evidence of Nephrotoxicity Based on Measured Creatinine Clearance

Bars = Min and Max
Refurbishing old antibiotic:  
2. Novel polymyxins * ?

- Colistin (Polymyxin E; discovered in 1949 and without clinical use for long) has now become the "last resource" antibiotics in the treatment of infections caused by multi-resistant organisms…

| Colistin: the revival of polymyxins for the management of multidrug-resistant gram-negative bacterial infections. |

- But colistin is a fairly toxic antibiotic (nephrotoxicity), which limits the concentrations that can be safely used, and therefore, limits its activity).
- Polmyxin B is more active but more toxic …
- Better compounds are badly needed, but the mode of action of colistin (membrane permabilization) should be retained because it ensures a fast bactericidal effect AND synergy with other antibiotics

* in collaboration with Northern Antibiotics, Finland
Colistin Microbiology: morphological aspects

Polymyxins synergy: the rationale (1)

- Gram-negative bacteria have also efflux systems defeating the passage of drugs across the OM and explaining the low activity of many antibiotics (intrinsic resistance) and the so-called "adaptative" resistance (aminglycosides)
Polymyxins synergy: the rationale (2)

- Disrupting the OM (as colistin does) will facilitate access of the other antibiotics to their targets
- This may apply EVEN to antibiotics for which the bacteria are resistant (if due to OM impermeability/efflux phenomenon)
### Novel polymyxin B derivatives

<table>
<thead>
<tr>
<th>Polymyxin B</th>
<th>MHAMOA</th>
<th>Dab&lt;sup&gt;+&lt;/sup&gt;</th>
<th>Thr</th>
<th>Dab&lt;sup&gt;+&lt;/sup&gt;</th>
<th>ri[Dab</th>
<th>Dab&lt;sup&gt;+&lt;/sup&gt;</th>
<th>pPhe</th>
<th>Leu</th>
<th>Dab&lt;sup&gt;+&lt;/sup&gt;</th>
<th>Dab&lt;sup&gt;+&lt;/sup&gt;</th>
<th>Thr</th>
</tr>
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<tbody>
<tr>
<td>NAB739</td>
<td>OA</td>
<td>-</td>
<td>Thr</td>
<td>-Dab&lt;sup&gt;+&lt;/sup&gt;</td>
<td>pSer</td>
<td>-ri[Dab</td>
<td>Dab&lt;sup&gt;+&lt;/sup&gt;</td>
<td>pPhe</td>
<td>Leu</td>
<td>Dab&lt;sup&gt;+&lt;/sup&gt;</td>
<td>Dab&lt;sup&gt;+&lt;/sup&gt;</td>
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<td>OA</td>
<td>-</td>
<td>Thr</td>
<td>-Abu</td>
<td>-ri[Dab</td>
<td>Dab&lt;sup&gt;+&lt;/sup&gt;</td>
<td>pPhe</td>
<td>Leu</td>
<td>Dab&lt;sup&gt;+&lt;/sup&gt;</td>
<td>Dab&lt;sup&gt;+&lt;/sup&gt;</td>
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<td>Ac</td>
<td>-</td>
<td>Thr</td>
<td>-pSer</td>
<td>-ri[Dab</td>
<td>Dab&lt;sup&gt;+&lt;/sup&gt;</td>
<td>pPhe</td>
<td>Leu</td>
<td>Dab&lt;sup&gt;+&lt;/sup&gt;</td>
<td>Dab&lt;sup&gt;+&lt;/sup&gt;</td>
<td>Thr</td>
</tr>
</tbody>
</table>

- The MIC<sub>90</sub> of NAB739 for *E. coli* and Enterobacteriaceae are similar to those of polymyxin B (1-2 mg/L).
- NAB739 is also active against *Acinetobacter baumannii*, and *Pseudomonas aeruginosa*.
- NAB7061 and NAB741 strongly synergize the activity of antibiotics (including rifampicin, macrolides, fusidic acid and vancomycin) towards Gram (-) pathogens.

NAB compounds are less cytotoxic than polymyxin B

LDH release (cytotoxicity) in cultures renal cells (LLC-PK1)

Mingeot-Leclercq et al. 51st Interscience Conference on Antimicrobial Agents and Chemotherapy, Chicago, IL, 2011
Refurbishing old antibiotics

3. Temocillin *

Temocillin revived

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Resistance in Gram-negative pathogens is an increasing concern, with carbapenems often appearing as the only acceptable treatment option in serious infections. Reviving older compounds that have fallen into disuse may help to alleviate this burden. Temocillin (5-α-methoxy-ticarcillin) is resistant to most if not all classical and extended-spectrum  β-lactamases and to AmpC enzymes. It is also chemically stable, allowing administration by continuous infusion. Pharmacokinetic/pharmacodynamic analysis, aided by Monte-Carlo simulations, suggests a breakpoint of 8 mg/L for the registered maximum dosage of 4 g daily. Temocillin’s weaknesses, explaining its limited previous use, are a lack of activity against Gram-positive organisms, anaerobes and *Pseudomonas*. In settings where these are unlikely or are covered by other agents, temocillin may be useful, potentially ‘sparing’ carbapenems and having little apparent potential to select for *Clostridium difficile*.

* in collaboration with Eumedica (Belgian SME)
Temocillin in a nutshell

The α-methoxy group (arrow) in temocillin blocks access of water (W1) to the active serine (S70) of β-lactamase, thereby blocking the chain of molecular events leading to hydrolysis.

Efflux and resistance

- Efflux is a universal mechanism for cell protection against "toxic" membrane-diffusing agents.
- Many drugs diffuse through membranes because we made them amphiphilic to favor their diffusibility … and become opportunistic substrates for efflux pumps.
- For AB, efflux decreases the amount of drug in bacteria and impairs activity, increasing the MIC …
- Insufficient drug exposure favors the selection of less sensitive organisms.

Why is temocillin not active against *P. aeruginosa*?

Table 1. MICs of temocillin and ticarcillin against *P. aeruginosa* strains with known expression of the efflux Mex components in Mueller-Hinton broth (MHB) and in MHB supplemented with the broad spectrum efflux transporter inhibitor Phe-Arg-β-naphthylamide (PAβN; 50 µg/mL)

<table>
<thead>
<tr>
<th>Strains</th>
<th>Origin or Ref.</th>
<th>Description</th>
<th>Expression of Efflux system</th>
<th>MIC (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Reference strain</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PAO1</td>
<td>ATCC</td>
<td>Reference strain</td>
<td>AB ^a XY ^a OprM ^a CD ^b EF ^b</td>
<td>Temocillin (+PAβN)</td>
</tr>
<tr>
<td><strong>Clinical isolates</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>d</td>
<td>3.97 9.04 ND + +</td>
<td></td>
<td>512 (128)</td>
</tr>
<tr>
<td>11</td>
<td>d</td>
<td>3.56 5.68 ND - -</td>
<td></td>
<td>&gt;512 (64)</td>
</tr>
<tr>
<td>156</td>
<td>d</td>
<td>0.33 0.95 ND - +</td>
<td></td>
<td>512 (64)</td>
</tr>
<tr>
<td>68</td>
<td>d</td>
<td>0.87 4.94 ND - -</td>
<td></td>
<td>&gt;512 (64)</td>
</tr>
<tr>
<td>333A</td>
<td>d</td>
<td>2.17 2.29 ND - -</td>
<td></td>
<td>&gt;1024 (1024)</td>
</tr>
<tr>
<td>34</td>
<td>d</td>
<td>6.66 1.26 ND - -</td>
<td></td>
<td>&gt;1024 (512)</td>
</tr>
<tr>
<td>168B</td>
<td>d</td>
<td>1.15 0.89 ND - -</td>
<td></td>
<td>256 (32)</td>
</tr>
<tr>
<td><strong>Engineered strains</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FB1</td>
<td>3</td>
<td>PAO1Δ(mexB::FRT)</td>
<td>ND ND ND ND ND ND</td>
<td>2</td>
</tr>
<tr>
<td>PAO1 mexAB</td>
<td>4</td>
<td>PAO1Δ(mexAB::FRT)</td>
<td>0e 1.08 ND - +</td>
<td>4 (2)</td>
</tr>
<tr>
<td>PAO200</td>
<td>4</td>
<td>PAO1Δ(mexAB-oprM)</td>
<td>0e 1.26 ND - -</td>
<td>4 (0.5)</td>
</tr>
<tr>
<td>CB536</td>
<td>5</td>
<td>PAO1Δ(mexCD-oprJ)</td>
<td>1.09 1.65 ND - +</td>
<td>128 (16)</td>
</tr>
<tr>
<td>CB603</td>
<td>5</td>
<td>PAO1Δ(mexEF-oprN)</td>
<td>1.21 1.06 0.51 - -</td>
<td>128 (32)</td>
</tr>
<tr>
<td>CB602</td>
<td>5</td>
<td>PAO1Δ(mexXY-oprM)</td>
<td>1.10 0.06 0.55 - +</td>
<td>64 (16)</td>
</tr>
<tr>
<td><strong>PAO1Δ(oprM)</strong></td>
<td></td>
<td>PAO1 Δ(oprM)</td>
<td>ND ND ND ND ND ND</td>
<td>2</td>
</tr>
<tr>
<td>4098</td>
<td>6</td>
<td>Clinical strain</td>
<td>1.26 1.62 0.33 - -</td>
<td>256 (128)</td>
</tr>
<tr>
<td>4098E</td>
<td>6</td>
<td>4098 overproducing OprM</td>
<td>5.41 1.31 3.19 - -</td>
<td>1024 (512)</td>
</tr>
<tr>
<td>4098ET</td>
<td>6</td>
<td>4098E Δ(oprM)</td>
<td>2.18 0.04 0.02 - -</td>
<td>2 (f)</td>
</tr>
</tbody>
</table>

---

^a Real-time PCR (threshold ratio compared to PAO1; values of ≥ 2 and 5 are considered to denote highly significant overexpression of mexAB and mexXY, respectively. ^b RT-PCR (qualitative detection [+ / -]). ^c Phe-Arg-β-naphthylamide (broad spectrum efflux inhibitor) used at 50 mg/L. ^d isolated from Intensive Care patients with a clinical diagnostic of health care-associated pneumonia. ^e complete absence of detection. ^f No growth; PAβN MIC = 25 mg/L.

Buyck et al. 51th ICCAC, Chicago, IL, 2011
Structure of antibiotic efflux transporters

RND, MFS, SMR  MATE  ABC  RND, MFS, ABC

in P. aeruginosa

Van Bambeke et al.
Using macrolides to block the synthesis of OprM in \textit{P. aeruginosa}

7 pillars of wisdom….

1. Public education
2. Public health, sanitation and quality of life
3. New antibiotics → new / poorly exploited targets
4. Old antibiotics
   → aminoglycosides – polymyxins - temocillin
5. **Better use of antibiotics**
   → PK/PD approaches against resistance
   → Intracellular bacteria
6. Alternatives to antibiotics
7. Collaborative approach
Pharmacokinetics/Pharmacodynamics of antibiotics

Concentration

\[ C_{\text{max}} \]

\[ \frac{C_{\text{max}}}{\text{MIC}} \]

\[ \frac{AUC_{24h}}{\text{MIC}} \]

\[ fT > \text{MIC} \]

Time (h)

0 6 12 18 24

MIC

AUC_{24h}
Avoiding selection of resistant mutants during treatment: an example with fluoroquinolones

\[ \text{AUC}_{24h} = \frac{\text{dose}_{24h}}{\text{clearance}} \]

Lack of resistance of *S. pneumoniae* to moxifloxacin over 10 years of large use in the community in Belgium

*S. pneumoniae* susceptibility to moxifloxacin in Belgium

From data of a national collection
- Non invasive respiratory tract infections
- similar results in 2008 for a collection of *S. pneumoniae* from clinically-confirmed CAP

Intracellular bacteria: setting up a model

pharmacological concentration-response curves

- Van Bambuke, unpublished
The difficulties in eradicating intracellular (hidden) bacteria: an example with \textit{P. aeruginosa}

7 pillars of wisdom….

1. Public education
2. Public health, sanitation and quality of life
3. New antibiotics → new / poorly exploited targets
4. Old antibiotics
   → aminoglycosides – polymyxins - temocillin
5. Better use of antibiotics
6. **Alternatives to antibiotics**
7. Collaborative approach
Alternatives to antibiotics

Inhibitors of type III secretion systems in *P. aeruginosa*

Anantharajah *et al.* ICAR 2012
(in collaboration with Creative Antibiotics, Umea, Sweden)

Stimulation of phagocytosis of *P. aeruginosa* by fully-human monoclonal antibody (panomacuab)

Jacqmin *et al.* ICAAC 2012
(in collaboration with Kenta Biotech, Zurich, Switzerland)
Towards medicine … and success?

Healing Buddha

The last Judgment
Hieronymus Bosch (c1450-1516)
Vienna Art Academy
Academic partnerships
Main Industrial partnerships for common projects *

* most having led to peer-reviewed publications on novel compounds or concepts
Collaborative approach to bring discovery to the clinics

University Clinic (900 beds)

Faculty (teaching and research)

Associated Institutions

Students' quarters

about 6,000 students in Health Sciences
Who made that all possible?
Who made that all possible?
Disclosures

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