From new bacterial targets to clinical applications

Paul M. Tulkens, MD, PhD

on behalf of the

Cellular and Molecular Pharmacology Group
Louvain Drug Research Institute
Université catholique de Louvain
Brussels, Belgium
Our main research interests...

- cellular pharmacokinetics
- cellular pharmacodynamics
- antibiotic toxicity
- resistance
- novel bacterial targets
- clinical applications

antibiotics: from molecules to man
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 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?
Resistance of *P. aeruginosa* in hospitals
(International data – EUCAST breakpoints)

Spreading of NDM-1 in the community …

Outbreak of Carbapenem-Resistant Enterobacteriaceae Containing $bla_{NDM-1}$
Ontario, Canada

Sergio Borgia1,2,4 Olga Lastovetska,4,5 David Richardson1,2,9 Alireza Eshaghi,4 Jianhui Xiong,3 Catherine Chung,6 Mahin Baqi,1,7 Allison McGeer,5,8 Gloria Ricci,2 Rachael Savicki,3 Rajni Pantelidis,3 Donald E. Low,4,5,6 Samir N. Patel4,5,9 and Roberto G. Molano1,5,9

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 8Infection Prevention and Control, Mount Sinai Hospital, Toronto, and Departments of 5,9Medicine and 5Pathology and Molecular Medicine, McMaster University, Hamilton, Ontario, Canada


NDM-1-Producing Klebsiella pneumoniae Resistant to Colistin in a French Community Patient without History of Foreign Travel

Corinne Arpin,4 Patrick Noury,4 Delphine Boraud,4 Laure Coulange,4 Alain Manetti,5 Catherine André,6 Fatima M’Zali,4 and Claudine Quentin4

Université de Bordeaux, Microbiologie Fondamentale et Pathogénicité UMR 5234, Bordeaux, France4; Laboratoire de Biologie Médicale EXALA, Site de Villenave-d’Ornon, Villenave d’Ornon, France5; and Agence Régionale de Santé, Espace Rodesse, Bordeaux, France4

A carbapenem-resistant 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 metallo-carbapenemase NDM-1 and was resistant to all antibiotics except tetracyclines and colistin. The K. pneumoniae strain belonged to sequence type ST15, and $bla_{NDM-1}$ was carried by a non-typeable conjugative plasmid. Two months later, a similar ST15 isolate, Kp5241, was present in the patient but was additionally colistin resistant.
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.)
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\(^a,b\), Narcisa Mesaros\(^a,3\), 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 Bambeke\(^a\), 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
“Father resistance genes”:
an original example with aminoglycosides

Aminoglycoside Antibiotic-Inactivating Enzymes in Actinomycetes Similar to Those Present in Clinical Isolates of Antibiotic-Resistant Bacteria
(streptomyces/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 fittest!
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 antri-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</th>
<th>TEM-exposed</th>
<th>Revertant</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MIC (mg/L) a</td>
<td>MIC (mg/L)</td>
<td>MIC (mg/L)</td>
</tr>
<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

<table>
<thead>
<tr>
<th>strains</th>
<th>Initial</th>
<th></th>
<th>TEM-exposed</th>
<th></th>
<th>Revertant</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MIC (mg/L)</td>
<td>TEM</td>
<td>FEP</td>
<td>MEM</td>
<td>MIC (mg/L)</td>
</tr>
<tr>
<td>2114/2</td>
<td>8</td>
<td>2</td>
<td>0.25</td>
<td>2048</td>
<td>&gt; 128</td>
</tr>
<tr>
<td>2502/4</td>
<td>8</td>
<td>2</td>
<td>0.125</td>
<td>8192</td>
<td>4</td>
</tr>
<tr>
<td>3511/1</td>
<td>32</td>
<td>2</td>
<td>0.125</td>
<td>4096</td>
<td>32</td>
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<tr>
<td>7102/10</td>
<td>512</td>
<td>32</td>
<td>1</td>
<td>16384</td>
<td>&gt; 128</td>
</tr>
</tbody>
</table>

* figures in bold indicate values > the R breakpoint for Enterobacteriaceae (EUCAST for MEM [8] and FEP [4]; BSAC and Belgium for TEM [16])

* dot blot 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

* ESBL TEM 24 (+); * ESBL (-) and AmpC (+) [high level]; * Intermediate (I) according to EUCAST

Nguyen et al. (post-doc at LDRI) presented at the 8th ISAAR, Seoul, Korea, on 8 April 2011 and additional work in progress.
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 $\rightarrow$ 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

\[
\text{D-Ala} + X \xrightarrow{\text{Ligase}} \text{D-Ala-D-X} \\
\text{UDP-} \text{L-Ala-D-Glu-L-Lys} \xrightarrow{\text{MurF}} \text{L-Ala-D-Glu-L-Lys-D-Ala-D-X} \\
\text{UDP-} \text{L-Ala-D-Glu-L-Lys-D-Ala-D-X} \xrightarrow{\text{pentapeptide}} \text{L-Ala-D-Glu-L-Lys-D-Ala-D-X}
\]
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>Semicarbazides</th>
<th><img src="image" alt="Chemical Structure" /></th>
</tr>
</thead>
<tbody>
<tr>
<td>17 molécules</td>
<td></td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>Triazoles</th>
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<tbody>
<tr>
<td>8 molécules</td>
<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Dioxo-Indolines</th>
<th><img src="image" alt="Chemical Structure" /></th>
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</thead>
<tbody>
<tr>
<td>7 molécules</td>
<td></td>
</tr>
</tbody>
</table>
Semi-carbazides are better ...

S89 is fairly active

---

### Family of Semi-carbazides

<table>
<thead>
<tr>
<th>Souche</th>
<th>Caractéristique et référence</th>
<th>CMI (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. aureus ATCC33591</em></td>
<td>HA-MRSA (Lemaire et al., 2008)</td>
<td>16</td>
</tr>
<tr>
<td><em>S. aureus NRS192</em></td>
<td>CA-MRSA (PV+) (Lemaire et al., 2008)</td>
<td>8</td>
</tr>
<tr>
<td><em>S. aureus NRS126</em></td>
<td>HA-MRSA/VISA (Lemaire et al., 2008)</td>
<td>16</td>
</tr>
<tr>
<td><em>S. aureus VR5-1</em></td>
<td>HA-MRSA/VRSA (type VanA) (Lemaire et al., 2008)</td>
<td>16</td>
</tr>
<tr>
<td><em>Streptococcus pneumoniae</em> ATCC49619</td>
<td>Souche sensible</td>
<td>32</td>
</tr>
<tr>
<td><em>Listeria monocytogenes EGD</em></td>
<td>Souche sensible (Duadhriri et al., 1999)</td>
<td>16</td>
</tr>
</tbody>
</table>

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**Graph**: Activity relative to the control (% of control) for various semi-carbazides tested at 0.6 mM in 10% DMSO.

**Table**: Activity of selected semi-carbazides against various bacterial strains, along with their CMI values.

**Notes**: *S89* is fairly active.
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 \(\rightarrow\) 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
MINIREVIEW

Aminoglycosides: Activity and Resistance

MARIE-PAULE MINGEOT-LECLERCOQ,† YOURI GLUPCZYNSKI,‡ 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,‡ 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).
Aminoglycosides: Nephrotoxicity

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

Unité de Pharmacologie Cellulaire et Moléculaire, Université Catholique de Louvain, Brussels, Belgium
Aminoglycoside nephrotoxicity

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, pericellular 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 58, 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\textsubscript{50}</th>
<th>MIC\textsubscript{90}</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>
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<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 Iothalamate 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
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...

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
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

- The MIC90 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

David M. Livermore1* and Paul M. Tulkens2

1Antibiotic Resistance Monitoring and Reference Laboratory, Health Protection Agency Centre for Infections, 61 Colindale Avenue, London NW9 5EQ, UK; 2Unité de Pharmacologie Cellulaire et Moléculaire & Centre de Pharmacie Clinique, Université Catholique de Louvain, Bruxelles, Belgium

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 (6-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.

**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>
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<td></td>
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<td>AB a XY a OprM a CD b EF b</td>
<td>Temocillin (+PAβN)</td>
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<td>4098 overproducing OprM</td>
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<td>4098E Δ(oprM)</td>
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</table>

* 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.
Structure of antibiotic efflux transporters

Macrolides inhibit the formation of OprM of *P. aeruginosa* in eucaryotic media

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

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

\[ fT > \text{MIC} \]

\[ \text{AUC}_{24h} / \text{MIC} \]
Avoiding selection of resistant mutants during treatment: an example with fluoroquinolones

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

<table>
<thead>
<tr>
<th>MIC (μg/mL)</th>
<th>0.007</th>
<th>0.0156</th>
<th>0.0312</th>
<th>0.0625</th>
<th>0.125</th>
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<tbody>
<tr>
<td>cumulative percentage</td>
<td>0%</td>
<td>25%</td>
<td>50%</td>
<td>75%</td>
<td>100%</td>
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</tbody>
</table>

- Surveys from the Belgian Scientific Institute for Public Health for *S. pneumoniae* from community isolates (n=156 in 1999 and 448 in 2008)
- Data available yearly for 1999 through 2008
- http://www.iph.fgov.be

The difficulties in eradicating intracellular (hidden) bacteria: an example with *P. aeruginosa*
7 pillars of wisdom....

1. Public education
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4. Old antibiotics
   → aminoglycosides – polymyxins - temocillin
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7. Collaborative approach
Alternatives to antibiotics

Inhibitors of type III secretion systems in P. aeruginosa

in collaboration with Creative Antibiotics, Umea, Sweden

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

in collaboration with Kenta Biotech, Zurich, Switzerland
Towards medicine … and success?

Yakushi-Nyorai
(healing Buddha)
To-ji 東寺, Kyoto 京都

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?

******** January 2013 ********
Disclosures

Financial support from

• the Belgian *Fonds de la Recherche Scientifique* (and other federal and regional funding agencies) for basic research on pharmacology and toxicology of antibiotics and related topics and for support to a PhD fellow (D. Das)

• The *Université catholique de Louvain* for personal support

• the Belgian Public Federal Service "Public Health" for "Appropriate antibiotic use" studies in General Practice

• The *Pôles d'Attraction Interuniversitaire/ Interuniversitair Attractie Polen* programme of the Belgian Federal Government, the *Région Bruxelloise/Brusselse Gewest* and the *Région Wallonne* for support to post-doctoral fellows

• Collaborations with Achaogen, Northern Antibiotics, RibX, Merlion, Trius, Cerexa, Bayer, AstraZeneca, and GSK.
Back-up slides
Rationale

- 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
Inhibition of purified His-tagged D-Ala – D-Ala ligase by D-cycloserine *

IC$_{50}$ = 1.55 mM

* broad but weak antimicrobial activity (through inhibition of D-Ala-racemase and, to a lesser extent, D-Ala-D-Ala ligase); used as 2d/3d line drug against M. tuberculosis (MIC: 5-20 mg/L; 50-200 μM); usage limited because of CNS toxicity

Literature data:
- 2 mM (Feng et al., AAC 2003;47:283-291)
- 0.8 mM (Park et al., PNAS 1997;94:10040-10044)
No Evidence of Nephrotoxicity Based on Daily Serum Creatinine

Bars = Min and Max
No Evidence of Nephrotoxicity Based on Daily BUN Measurements

Bars = Min and Max
No Evidence of Nephrotoxicity Based on Measured Creatinine Clearance

Bars = Min and Max
Colistin Microbiology: morphological aspects

Colistin Microbiology: morphological aspects

Live *Acinetobacter baumanii* as seen in Atomic Force Microscopy (AFM°)

Untreated | Colistin 1 mg/L | Colistin 32 mg/L
---|---|---
A | B | C

- **susceptible**
- **resistant**

Efflux and resistance

• efflux is a universal mechanism for cell protection against "toxic" membrane-diffusing agents
• many drugs diffuse though 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

Van Bambeke et al.  
Structure of antibiotic efflux transporters

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

Gram (-) transporters (incl. mex pumps)

Structure-based approach

Example with an ABC transporter

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

Van Bambeke et al.
Antibiotic resistance: short overview of main mechanisms

- Wild strain
- Antibiotic inactivation (biotransformation)
- Target modification
- Alternative target or multiplication of the target
- Impermeabilization
- Efflux pump

1. Active antibiotic
2. Inactive antibiotic
3. Useless antibiotic
4. Surpassed antibiotic
5. Reduced amount of antibiotic
6. Reduced amount of antibiotic
The situation in the community: 
*S. pneumoniae* and macrolides in Europe (2001)
Structure-based approach

Dynamics and Structural Changes Induced by ATP Binding in SAV1866, a Bacterial ABC Exporter

Jean-Paul Becker,† Françoise Van Bambeke,‡ Paul M. Tulkens,‡ and Martine Prévost*†

Structure et Fonction des Membranes Biologiques, Université Libre de Bruxelles, Boulevard du Triomphe CP 206/2, B-1050 Brussels, Belgium, and Unité de Pharmacologie cellulaire et moléculaire, Université catholique de Louvain, Avenue E. Mounier 73, B-1200 Brussels, Belgium

Received: April 28, 2010; Revised Manuscript Received: September 6, 2010

ATP Binding in SAV1866

Figure 1. Ribbon representation depicting the crystal structure of the SAV1866 dimer (PDB code: 2ONJ). One monomer is green, the second monomer is white. The ATP molecules located in the NBDs are represented as sticks and are colored according to the atom represented (carbon in cyan, nitrogen in blue, oxygen in red, and phosphorus in bronze). ECL and CH stand for the extracellular loops and the intracellular coupling helices. The enlarged view shows a close-up illustration of one of the NBDs and of the main motifs common to members of the ABC exporter family.
Structure-based approach: the substrate

Figure 3. Dynamics of the internal TMD chamber in the nucleotide-free and the nucleotide-bound trajectories. Access paths originating at the bottom center of the TMD identified with the MOLE program at 0, 40, and 80 ns of the simulation time. Upper: In the nucleotide-free trajectory. (A) At 0 ns a central path is identified that eventually splits at its extracellular extremity. (B) At 40 ns a central path persists, albeit with a much smaller radius. (C) At 80 ns, no central cavity exiting to the extracellular medium is identified anymore. Other, narrower paths exit to the cytosol and inner membrane leaflet. Lower: In the nucleotide-bound trajectory. (D) and (E) At 40 and 80 ns a central tunnel is observed. The 0 ns conformation is not shown as it is identical to that in the nucleotide-bound trajectory.
From ciprofloxacin to finafloxacain…

ciprofloxacin

avoids efflux

moxifloxacain
(Bayer HealthCare)

lowers MIC

finafloxacain

from ciprofloxacin to finafloxacain
The connectome….
(cross-resistance)

http://wrightlab.mcmasteriidor.ca/
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) …