Efflux as a new challenge in antibacterial chemotherapy

Paul M. Tulkens
Unité de pharmacologie cellulaire et moléculaire
Université catholique de Louvain, Bruxelles

Transport across membranes: Multiple drug resistance, mechanisms and new tools
Bremen, Germany, July 9th, 2007, … in the evening

Influenced in large part by my active participation to the European Committee for Antibiotic Susceptibility Testing (EUCAST) and to the International Society of Antiinfective Pharmacology (ISAP)
You said "Challenge"?
You said "Challenge"?

**challenge (INVITATION) */"tʃɛn.ɪ.dʒ/ noun [C]**

- an invitation to compete or take part, esp. in a game or argument
  - "I bet you can't eat all that food that you've got on your plate." "Is that a challenge?"
  - Is there going to be a challenge for the position of chairperson when the next election for the committee is held?
  - She issued a challenge to her rival candidates to take part in a public debate, but they did not accept it.
You said "Challenge"?

**challenge** (INVITATION)  /"tʃɛllɪndʒ/ noun [C]

- an invitation to compete or take part, esp. in a game or argument

- "I bet you can't eat all that food that you've got on your plate." "Is that a challenge?"

- Is there going to be a challenge for the position of chairperson when the next election for the committee is held?

- She issued a challenge to her rival candidates to take part in a public debate, but they did not accept it.

*the hungry microbiologist*
You said "Challenge"?

**challenge** (INVITATION) /"tʃɛlɪndʒ/ noun [C]

- an invitation to compete or take part, esp. in a game or argument
  - "I bet you can't eat all that food that you've got on your plate." "Is that a challenge?"
  - Is there going to be a challenge for the position of chairperson when the next election for the committee is held?
  - She issued a challenge to her rival candidates to take part in a public debate, but they did not accept it.

---

for the next conference on efflux
You said "Challenge"?

**challenge (INVITATION) /"tʃɛləndʒ/ noun [C]**

- an invitation to compete or take part, esp. in a game or argument
  - "I bet you can't eat all that food that you've got on your plate." "Is that a challenge?"
  - Is there going to be a challenge for the position of chairperson when the next election for the committee is held?
  - She issued a challenge to her rival candidates to take part in a public debate, but they did not accept it.

Are you talking about a political person here?
Challenges of efflux in antibacterial chemotherapy

• **recognizing its existence:** is it a major and general means of resistance?

• **its role:** does it need to change our vision on (and decisions about) existing antibiotics?

• **the way we find it:** how can we detect it (and do we need to do this?)

• **the way we treat patients:**
  – can we make non-effluxed drugs?
  – and what about efflux inhibitors?
  – is efflux important in pharmacokinetics/drug interactions?
Challenges of efflux in antibacterial chemotherapy

- **recognizing its existence:** is it a major and general means of resistance?
- **its role:** does it need to change our vision on (and decisions about) existing antibiotics?
- **the way we find it:** how can we detect it (and do we need to do this?)
- **the way we treat patients:**
  - can we make non-effluxed drugs?
  - and what about efflux inhibitors?
  - is efflux important in pharmacokinetics/drug interactions?
Historical observations on tetracyclines ...

IZAKI K, ARIMA K.

PMID: 14087909 [PubMed - indexed for MEDLINE]

Who remembers that car?
Historical observations on tetracyclines …

DISAPPEARANCE OF OXYTETRACYCLINE ACCUMULATION IN THE CELLS OF MULTIPLE DRUG-RESISTANT ESCHERICHIA COLI.

IZAKI K, ARIMA K.
PMID: 14087909 [PubMed - indexed for MEDLINE]

Who remembers that graph?
Resistance of *Escherichia coli* to Tetracyclines

BY T. J. FRANKLIN AND A. GODFREY

*Imperial Chemical Industries Ltd. (Pharmaceuticals Division), Alderley Park, Macclesfield, Cheshire*

*(Received 23 March 1964)*

1. A strain of *Escherichia coli* highly resistant to chlorotetracycline and partially cross-resistant to tetracycline has been isolated. 2. The nitro-reductase system of the resistant cells was inhibited to a smaller extent by chlorotetracycline than was the corresponding enzyme of sensitive cells. 3. The incorporation of leucine *in vitro* into the ribosomal protein of cell-free preparations from sensitive and resistant cells was equally inhibited by chlorotetracycline. 4. Resistant cells accumulated much less chlorotetracycline and tetracycline than did sensitive cells when both were cultured in the presence of these drugs. 5. The uptake of tetracycline by both sensitive and resistant *E. coli* was dependent on the presence of glucose in the medium. 6. Fractionation of cells cultured in medium containing [14C]chlorotetracycline indicated that the largest proportion of radioactivity in sensitive cells was in the fraction consisting mainly of cell-wall material. There was no concentration of radioactivity in any one fraction of the resistant cells. 7. No evidence could be obtained for a specific tetracycline-excretion system in the resistant cells. 8. The significance of these results in relation to current theories of the antibiotic action of and resistance to the tetracycline drugs is discussed.
Historical observations on tetracyclines ...
Historical observations on tetracyclines ...

Biochem. J. (1965) 94, 54

Resistance of *Escherichia coli* to Tetracyclines

By T. J. FRANKLIN and A. GODFREY
Imperial Chemical Industries Ltd. (Pharmaceuticals Division), Alderley Park, Macclesfield, Cheshire

(Received 23 March 1964)

1. A strain of *Escherichia coli* highly resistant to chlortetracycline and partially cross-resistant to tetracycline has been isolated. 2. The nitro-reductase system of the resistant cells was inhibited to a smaller extent by chlortetracycline than was the corresponding enzyme of sensitive cells. 3. The incorporation of leucine *in vitro* into the ribosomal protein of cell-free preparations from sensitive and resistant cells was equally inhibited by chlortetracycline. 4. Resistant cells accumulated much less chlortetracycline and tetracycline than did sensitive cells when both were cultured in the presence of these drugs. 5. The uptake of tetracycline by both sensitive and resistant *E. coli* was dependent on the presence of glucose in the medium. 6. Fractionation of cells cultured in medium containing [14C]chlortetracycline indicated that the largest proportion of radioactivity in sensitive cells was in the fraction consisting mainly of cell-wall material. There was no concentration of radioactivity in any one fraction of the resistant cells. 7. No evidence could be obtained for a specific tetracycline-excretion system in the resistant cells. 8. The significance of these results in relation to current theories of the antibiotic action of and resistance to the tetracycline drugs is discussed.
Historical observations on tetracyclines …

Proc. Natl. Acad. Sci. USA
Biochemistry

Active efflux of tetracycline encoded by four genetically different tetracycline resistance determinants in Escherichia coli
(everted membrane vesicles/tetracycline transport/transposon Tn10/plasmids)

Laura McMurry, Richard E. Petrucci, Jr., and Stuart B. Levy*
Department of Molecular Biology and Microbiology and Department of Medicine, Tufts University School of Medicine, Boston, Massachusetts 02111

Communicated by Boris Magasanik, April 21, 1980
Historical observations on tetracyclines ...

**FIG. 1.** Tetracycline uptake by *E. coli* ML308-225 (sensitive) and by R222-containing induced (resistant) cells with (O) and without (●) 1 mM DNP. Cells were grown overnight in medium A containing glucose and uptake was measured in the absence of added energy source.

**FIG. 2.** Tetracycline (Tc) uptake by everted membrane vesicles made from sensitive ML308-225 cells and from uninduced and induced R222-containing cells. O, No energy; ●, D-lactate. Cells were grown in glycerol and vesicles were frozen in 5 mM Tris-HCl, pH 7.2/70 mM KCl/0.25 mM dithiothreitol/50% glycerol. The assay was done at pH 6.6.

McMurry et al., PNAS 1980; 77:3974-3977
Historical trends ...

No. of publications in PubMed with keywords: antibiotic & efflux
Historical landmarks …

Successive description of efflux-mediated resistance for major antibiotics

<table>
<thead>
<tr>
<th>Year</th>
<th>No. of publications / 2 years period</th>
</tr>
</thead>
<tbody>
<tr>
<td>1960</td>
<td>0</td>
</tr>
<tr>
<td>1970</td>
<td>50</td>
</tr>
<tr>
<td>1980</td>
<td>150</td>
</tr>
<tr>
<td>1990</td>
<td>250</td>
</tr>
<tr>
<td>2000</td>
<td>400</td>
</tr>
<tr>
<td>2010</td>
<td>450</td>
</tr>
</tbody>
</table>

- aminoglycosides
- rifampin
- β-lactams
- macrolides
- fluoroquinolones
- tetracyclines
- linezolid
Challenges …

• Efflux has, slowly but surely, been shown to affect most if not all major antibiotic classes …

? Are they classes that will never show efflux-mediated resistance (glycopeptides [vancomycin…], lipoglycopeptides [telavancin], lipopeptides [daptomycin], …)

? Is efflux taken in full consideration in present teaching not only of microbiology but in everyday clinical practice (including in clinical microbiology reports) and is understood by clinicians ?
Challenges of efflux in antibacterial chemotherapy

• **recognizing its existence**: is it a major and general means of resistance?

• **its role**: does it need to change our vision on (and decisions about) existing antibiotics?

• **the way we find it**: how can we detect it (and do we need to do this?)

• **the way we treat patients**:
  – can we make non-effluxed drugs?
  – and what about efflux inhibitors?
  – is efflux important in pharmacokinetics/drug interactions?
Challenges …

- Why is efflux still so poorly taken into account in teaching, in textbooks, and, in general, in medical education and practice?
  - efflux most often causes only low levels of resistance, which tends to make it "clinically insignificant" … (see more about that later on)

- Bacteria carrying the gene encoding macrolide efflux (i.e. the mefE gene) display relatively low-level resistance. Azithromycin, because of its ability to achieve concentrations at sites of infections, is capable of eradicating mefE-carrying strains.

Challenges …

• Why is efflux so poorly taken into account in teaching, in textbooks, and, in general, in medical education?
  ➢ the multiplicity of structurally and pharmacologically unrelated substrates make efflux hard to grasp and difficult to enter in specific chapters or sections

INTRODUCTION

Antibiotic resistance can be divided into six basic groups depending on the mechanism involved:

• the presence of an enzyme that inactivates the antibiotic;
• the presence of an alternative enzyme for that inhibited by the antibiotic;
• mutation in the target, which reduces binding of the antibiotic to the target;
• modification of the target, which reduces binding of the antibiotic to the target;
• reduced uptake of the antibiotic; and
• active efflux of the antibiotic.
Challenges …

• Why is efflux so poorly taken into account in teaching, in textbooks, and, in general, in medical education?

  the multiplicity of pumps with "hard to understand names" does not help…

<table>
<thead>
<tr>
<th>Famille (dénomination*)</th>
<th>Acronyme</th>
<th>Source d’énergie</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATP-Binding Cassette</td>
<td>ABC</td>
<td>ATP</td>
</tr>
<tr>
<td>Major Facilitator Superfamily</td>
<td>MFS</td>
<td></td>
</tr>
<tr>
<td>Resistance-Nodulation-cell Division</td>
<td>RND</td>
<td>Gradients d’ions</td>
</tr>
<tr>
<td>Small Multidrug Resistance</td>
<td>SMR</td>
<td></td>
</tr>
</tbody>
</table>

* Les dénominations sont celles données lors de la découverte des transporteurs, et correspondent souvent à des éléments contingents liés à cette découverte, ce qui explique qu’ils ne soient pas nécessairement en rapport avec les fonctions établies sur la base des travaux ultérieurs.
Challenges …

• The real challenges are about educating microbiologists, clinicians and responsible persons about the real impacts of efflux
  
  ➢ efflux cooperates with other resistance mechanisms to move towards high resistance phenotypes
  
  ➢ efflux favours the emergence of "first-mutants" by subjecting bacterial targets to suboptimal concentrations
  
  ➢ efflux causes an overall decrease in antibiotic efficacy
Emergence of first mutants: the MPC concept...

"Classic" bactericidal effect

Surviving bacteria

concentration

$\text{MIC}_{99} = 0.8$

poorly sensitive organisms...

Elimination of resistant organisms

$\text{MPC}_{10} = 9$

Dong et al; AAC 43:1756-1758
Mutant Prevention Concentration ...

Concentration which will inhibit the majority of the organisms

Concentration needed to prevent the selection of resistant organisms

\[ \text{MIC}_{99} = 0.8 \]

\[ \text{MPC}_{10} = 9 \]

Dong et al; AAC 43:1756-1758
Mutant Prevention Concentration of ciprofloxacin and levofloxacin in *P. aeruginosa* (clinical isolates) with "normal" susceptibility (MIC = 0.33 and 0.9 mg/L) …

Mutant Prevention Concentration of ciprofloxacin and levofloxacin in *P. aeruginosa* (clinical isolates) with "normal" susceptibility (MIC = 0.33 and 0.9 mg/L) …

First mutants or efflux?

Levofloxacin and pneumococci in the World

Levofloxacin / Streptococcus pneumoniae
Antimicrobial wild type distributions of microorganisms – reference database EUCAST

Wild type distribution according to EUCAST

MIC
Epidemiological cut-off: WT ≤ 2 mg/L
Clinical breakpoints: S ≤ 2 mg/L, R > 2 mg/L

18405 observations (10 data sources)
Levofloxacin and pneumococci in Belgium

Levofloxacin MIC distributions in Belgian isolates

- In original surveys of levofloxacin susceptibility in the mid 90's, most MICs were $\leq 0.25$ mg/L
- Most Belgian isolates with MIC $> 0.25$ mg/L are carrying weak (in terms of MIC change) but clearly detectable reserpine-sensitive transporter(s)
- the limit of efficacy for the most commonly used dosage (500 mg) is 0.7 mg/L
- the presence of an efflux does contributes in reducing levofloxacin usefulness...

A. Lismond et al., unpublished
Fluoroquinolones and *P. aeruginosa* at an Academic Hospital in Belgium

---

**Limit of usefulness of levofloxacin (PK/PD)**

**Efflux or first mutants, or both?**

---

**J. van Eldere, 2003**
Challenges

• Efflux is like QnR for quinolones or arm for aminoglycosides: it is "before our nose" and "known by experts" but not much beyond that…

• the challenge is, therefore to clearly and unambiguously detect efflux in poorly sensitive isolates…
Challenges in Diagnostic

• Thesis:
  Efflux will continue to be largely ignored (and go undetected) as long as clinical microbiologists swear only (sorry, report susceptibility) by breakpoints only, derived from "classical" antibiograms made with the antibiotics they use in their hospital...

• Action:
  Since breakpoints will continue to exist and to be used, efflux needs to be detected by additional techniques to provide added-on warning…
You said "breakpoints"?

- a magic number derived from *in vitro* susceptibility testing, and used by the clinical microbiologists to tell the clinician whether the antibiotic will work, could work, or will fail with his/her **patient**.

- this number is usually derived from the measurement of a diameter of growth inhibition in an agar plate around a disk loaded with a standard amount of antibiotic;

- while what is measured is *per definition* a continuous variable (i.e. a diameter of any size [from 0 mm to the limit of the dish...]), microbiologists and authorities like to cut the results into 3 discrete categories:
  - less than x mm ➔ RESISTANT
  - larger than y mm ➔ SUSCEPTIBLE
  - between x and y ➔ INTERMEDIATE

which is what the clinician will get...

---

1 may be converted into an MIC (see later); automatic machines use growth rates...
Why do we use breakpoints?

To be honest, I always wondered …
Why do we need breakpoints?

but perhaps...

1. Doctors like to know if the bug is "good" or "bad" ...

2. Regulators like to tell people "DO" or "Don't"

3. Industry likes to know "When can I" and "When I cannot"

4. Lawyers like you to be "guilty" or "innocent" ...

5. Microbiologists wish to give them all simple answers...
Simple answers …

- Good !!
- Bad !!
- May be?
But, what is good?
But, what is bad?

Still Easy...

Good!!

Bad!!

serum concentration

MIC (µg/ml)

0.015 0.03 0.06 0.12 0.25 0.5 1 2 4 8 16 32
How…

No longer so easy…

serum concentration

Efflux?
The real question is how far above the "wild type" level can the bacteria MIC go and ... the patient still being treatable by the antibiotic ....
MIC data extracted from the MYSTIC database (http://www.mystic-data.org/) but limited to European countries; breakpoints are from EUCAST (http://www.eucast.org)
Application for Pseudomonas…

but this is also a risky zone…

MIC data extracted from the MYSTIC database (http://www.mystic-data.org/) but limited to European countries; breakpoints are from EUCAST (http://www.eucast.org)
Diagnostic approaches …

doi:10.1093/jac/dkl504
Advance Access publication 8 February 2007

A combined phenotypic and genotypic method for the detection of Mex efflux pumps in Pseudomonas aeruginosa

Narcisa Mesaros¹, Youri Glupczynski², Laëtitia Avrain¹, Nancy E. Caceres¹, Paul M. Tulkens¹* and Françoise Van Bambeke¹

¹Unité de Pharmacologie cellulaire et moléculaire, Brussels, Université catholique de Louvain, UCL 7370 avenue E. Mounier 73, B-1200 Bruxelles, Belgium; ²Laboratoire de Microbiologie, Cliniques universitaires UCL de Mont-Godinne, avenue G. Therasse 1, B-5530 Yvoir, Belgium
Diagnostic approaches …

Correlation between the level of expression (PCR) of constitutive Mex pumps and the effect of PAβN on the MIC of reporter antibiotics (carbenicillin for mexA and gentamicin for mexX).

Data are grouped in two quadrants of potentially different diagnostic significance:

- lower left, no or minimally meaningful efflux-mediated decrease of susceptibility
- upper right, efflux is likely to be the cause of the decreased susceptibility.

Diagnostic approaches …

• Tests must be simple but also as accurate as possible…
  – Genomic techniques are being rapidly introduced in the clinical laboratory and can either be automated (PCR) or made into fast-test assays
  – Accurate phenotypic and genotypic tests could be combined (E-test combined with mRNA detection)
  – Proteomic tests (using antibody-based detection techniques) could be added also.
A key to success ...

Pathology and epidemiology

Knowledge or ou “educated” suspicion of the causative agent

Is the organism probably highly susceptible?

yes

Local MIC data

Get an MIC

and get information about efflux

Recommend dosage adjustment on MIC and drug choice on efflux pattern

no

Recommend common dosage with PK/PD ...

9th July 2007  Bremen - Transport across membranes 46
Now the clinician may know ...

- **Wild type**
- **1st mutant**
- **Efflux QnR Porin, ...**
- **Combination**

**MIC**
Epidemiological cut-off: WT ≤ 0.064 mg/L
Clinical breakpoints: S ≤ 0.5 mg/L, R > 1 mg/L

6423 observations (9 data sources)
# Better breakpoints in Europe

## Fluoroquinolones - EUCAST clinical MIC breakpoints

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Ciprofloxacin</td>
<td>RD</td>
<td>0.5/1</td>
<td>0.5/1</td>
<td>1/1$^d$</td>
<td>1/1$^5$</td>
<td>--</td>
<td>--</td>
<td>0.125/2</td>
<td>0.5/0.5$^f$</td>
<td>0.03/0.06</td>
<td>0.03/0.06</td>
<td>--</td>
<td>0.5/1</td>
<td></td>
</tr>
<tr>
<td>Levofoxacin</td>
<td>RD</td>
<td>1/2</td>
<td>1/2</td>
<td>1/2</td>
<td>1/2</td>
<td>--</td>
<td>1/2</td>
<td>2/2</td>
<td>1/1$^f$</td>
<td>IE</td>
<td>IE</td>
<td>--</td>
<td>--</td>
<td>1/2</td>
</tr>
<tr>
<td>Moxifloxacin</td>
<td>RD</td>
<td>0.5/1</td>
<td>--</td>
<td>--</td>
<td>0.5/1</td>
<td>--</td>
<td>0.5/0.5$^f$</td>
<td>0.5/0.5$^l$</td>
<td>IE</td>
<td>IE</td>
<td>IE</td>
<td>0.5/1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Norfloxacin</td>
<td>RD</td>
<td>0.5/1</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>0.5/1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ofloxacin</td>
<td>RD</td>
<td>0.5/1</td>
<td>--</td>
<td>--</td>
<td>1/1$^3$</td>
<td>--</td>
<td>0.125/4</td>
<td>0.5/0.5$^f$</td>
<td>0.12/0.25</td>
<td>IE</td>
<td>--</td>
<td>0.5/1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1. Non-species related breakpoints have been determined mainly on the basis of PK/PD data and are independent of MIC distributions of specific species. They are for use only for species that have not been given a species-specific breakpoint and not for those species where susceptibility testing is not recommended (marked with -- or IE in the table).
2. For breakpoints for other fluoroquinolones (e.g. pefloxacin and enoxacin) - refer to breakpoints determined by national breakpoint committees.
3. *Salmonella* spp. - there is clinical evidence for ciprofloxacin to indicate a poor response in systemic infections caused by *Salmonella* spp. with low-level fluoroquinolone resistance (MIC>0.064 mg/L). The available data relate mainly to *S.*typhimurium but there are also case reports of poor response with other *Salmonella* species.
4. The S/A breakpoint has been increased from 0.5 to 1 mg/L to avoid dividing the wild type MIC distribution. Thus there is no intermediate category for *Acinetobacter* species.
5. *Staphylococcus* spp. - breakpoints for ciprofloxacin and ofloxacin relate to high dose therapy.
6. *Streptococcus pneumoniae* - wild type *S.pneumoniae* are not considered susceptible to ciprofloxacin or ofloxacin and are therefore categorized as intermediate. For ofloxacin the IR breakpoint was increased from 1.0 to 4.0 mg/L and for levofloxacin the S/A breakpoint from 1.0 to 2.0 to avoid dividing the wild type MIC distribution. The breakpoints for levofloxacin relate to high dose therapy.
7. Strains with MIC values above the S/A breakpoint are very rare or not yet reported. The identification and antimicrobial susceptibility tests on any such isolate must be repeated and if the result is confirmed the isolate sent to a reference laboratory. Until there is evidence regarding clinical response for confirmed isolates with MIC above the current resistant breakpoint (in italics) they should be reported resistant.
8. *Haemophilus influenzae* - fluoroquinolone low-level resistance (ciprofloxacin MIC’s of 0.125 - 0.5 mg/L) may occur in *H.influenzae*. There is no evidence that low-level resistance is of clinical importance in respiratory tract infections with *H.influenzae*.

$^a$ = Susceptibility testing not recommended as the species is a poor target for therapy with the drug.
$^b$ = There is insufficient evidence that the species in question is a good target for therapy with the drug.
$^d$ = Rationale: document listing data used for setting EUCAST breakpoints.

---

http://www.eucast.org
But we may do better: defining “safety” breakpoints

<table>
<thead>
<tr>
<th>Drug</th>
<th>Typical daily dosage(^a)</th>
<th>Typical PK values</th>
<th>Proposed PK/PD upper limit of sensitivity ((\mu)g/ml) for</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(C_{\text{max}}) in mg/L</td>
<td>AUC(_{24}\h) (mg (\times) h/L)</td>
</tr>
<tr>
<td>Norfloxacin</td>
<td>800 mg</td>
<td>1.4/1.1 (400 mg PO)</td>
<td>14/11</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>1000 mg</td>
<td>2.5/1.75 (500 mg PO)</td>
<td>24/18</td>
</tr>
<tr>
<td>Ofloxacin</td>
<td>400 mg</td>
<td>4/3 (400 mg PO)</td>
<td>40/30</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>500 mg</td>
<td>4/2.8 (500 mg PO)</td>
<td>40/28</td>
</tr>
<tr>
<td>Moxifloxacin</td>
<td>400 mg</td>
<td>3.1/1.8 (400 mg PO)</td>
<td>35/21</td>
</tr>
</tbody>
</table>

Challenges…

Will diagnostic solve everything?

- Level of efflux expression *in vivo*?
- How can dosage adjustment help?
- Combination of antibiotics?
- Tissue accumulation of antibiotics?
- Influence of other drugs that may share the same transporter(s)
Challenges of efflux in antibacterial chemotherapy

• **recognizing its existence:** is it a major and general means of resistance?

• **its role:** does it need to change our vision on (and decisions about) existing antibiotics?

• **the way we find it:** how can we detect it (and do we need to do this?)

• **the way we treat patients:**
  – can we make non-effluxed drugs?
  – and what about efflux inhibitors?
  – is efflux important in pharmacokinetics/drug interactions?
Challenges of efflux in antibacterial chemotherapy

• the way we treat patients:
  – can we make non-effluxed drugs?

Thesis:
Yes you can if you specifically aim at OR if you exploit what good nature (or good chemists) give you, but be prepared for partial successes …
Tigecycline...

- truly made to resist efflux-mediated resistance in Gram(-) bacteria
- broad spectrum including MRSA (MIC < 2 mg/L) and VISA
- tet(M) [ribosomal protection] or tet(K) [efflux] have no discernible effect on MICs (AAC 2006 Feb;50(2):505-10).
- approved by the FDA in 2005 and by the EMEA in 2006 for use in patients with complicated skin infections, skin-structure infections and intra-abdominal infections
### Origin of Tigecyclin

<table>
<thead>
<tr>
<th>Compound 2</th>
<th>Compound 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>(13-((3-chloropropyl)thio)-5-hydroxy-6-deoxytetracycline), a strong inhibitor of efflux but with reduced antibacterial activity</td>
<td>(9-N,N-dimethylglycylamido-minocyclin), a minocycline with enhanced activity</td>
</tr>
</tbody>
</table>

Two critical steps towards the discovery of efflux-resistant antibiotics maintaining antibacterial efficacy:

- Synthesis of the **hydrophobic** compound (2) from doxycycline by Levy and co-workers at the Center for Adaptation Genetics and Drug Resistance (Tufts University, School of Medicine, Boston, Mass.) revealed an inhibitory effect on tetracycline efflux (Nelson et al., 1994; J Med Chem 37: 1355-1361).

- Synthesis of compound (4) from minocycline (additional aminogroup slightly away from position 9) by Sum and colleagues at Chemical Sciences Laboratories (Wyeth-Ayerst Research, Pearl River, New York) showed that the activity could be improved (Sum et al. 1994; J Med Chem 37: 184-188).
The final selection of tigecycline was the result of a systematic research to combine the hydrophobic moiety AND the additional aminogroup in a substituent attached to position 9.

Table 2. In Vitro Antibacterial Activity of Compounds 13–25.

<table>
<thead>
<tr>
<th>R&lt;sub&gt;1&lt;/sub&gt;</th>
<th>E. coli UBMS 88-1 Tet B</th>
<th>E. coli PRP1 Tet A</th>
<th>E. coli J3272 Tet C</th>
<th>E. coli J3272 Tet D</th>
<th>E. coli UBMS 90-4 Tet M</th>
<th>S. aureus UBMS 88-7 Tet K</th>
<th>S. aureus UBMS 90-1 sensitive</th>
<th>S. aureus Smith sensitive</th>
<th>Enterococcus ATCC 29212</th>
</tr>
</thead>
<tbody>
<tr>
<td>13 MeNH</td>
<td>1</td>
<td>16</td>
<td>8</td>
<td>0.5</td>
<td>NT</td>
<td>1</td>
<td>16</td>
<td>1</td>
<td>0.5</td>
</tr>
<tr>
<td>14 n-PhNH</td>
<td>0.5</td>
<td>2</td>
<td>0.5</td>
<td>0.12</td>
<td>0.25</td>
<td>0.5</td>
<td>2</td>
<td>0.5</td>
<td>0.25</td>
</tr>
<tr>
<td>15 n-BuNH</td>
<td>0.5</td>
<td>1</td>
<td>0.5</td>
<td>0.25</td>
<td>0.25</td>
<td>0.5</td>
<td>2</td>
<td>0.5</td>
<td>0.25</td>
</tr>
<tr>
<td>16 t-BuNH</td>
<td>0.5</td>
<td>0.25</td>
<td>0.25</td>
<td>0.12</td>
<td>0.12</td>
<td>0.25</td>
<td>0.5</td>
<td>0.12</td>
<td>0.25</td>
</tr>
<tr>
<td>17 n-HexylNH</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.12</td>
<td>0.25</td>
<td>0.25</td>
<td>2</td>
<td>0.25</td>
<td>0.06</td>
</tr>
<tr>
<td>18 UndecylNH</td>
<td>32</td>
<td>32</td>
<td>32</td>
<td>32</td>
<td>32</td>
<td>16</td>
<td>2</td>
<td>16</td>
<td>0.5</td>
</tr>
<tr>
<td>19</td>
<td>4</td>
<td>32</td>
<td>8</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>4</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>20</td>
<td>0.25</td>
<td>1</td>
<td>0.25</td>
<td>0.12</td>
<td>0.25</td>
<td>0.25</td>
<td>2</td>
<td>0.25</td>
<td>0.12</td>
</tr>
<tr>
<td>21</td>
<td>4</td>
<td>2</td>
<td>2</td>
<td>0.5</td>
<td>2</td>
<td>4</td>
<td>0.5</td>
<td>1</td>
<td>0.25</td>
</tr>
<tr>
<td>22</td>
<td>0.5</td>
<td>1</td>
<td>0.5</td>
<td>0.25</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>23</td>
<td>0.5</td>
<td>4</td>
<td>0.5</td>
<td>0.25</td>
<td>0.5</td>
<td>0.5</td>
<td>4</td>
<td>1</td>
<td>0.5</td>
</tr>
<tr>
<td>24</td>
<td>2</td>
<td>4</td>
<td>2</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>2</td>
<td>0.5</td>
<td>0.25</td>
</tr>
<tr>
<td>25</td>
<td>16</td>
<td>32</td>
<td>16</td>
<td>8</td>
<td>8</td>
<td>16</td>
<td>32</td>
<td>8</td>
<td>4</td>
</tr>
</tbody>
</table>
Tigecycline is significantly modified compared to minocycline
Here is the "next in the pipeline" of the tetracylines "insensitive to efflux-mediated resistance"

MK-2764/PTK 0796 - the most advanced compound from a new class called the aminomethylcyclines (AMC) were derived from the tetracyclines. … an oral and IV once-daily antibiotic agent with activity against resistant and susceptible gram-positive, gram-negative, atypical and anaerobic bacteria… target community infections of the skin and respiratory tract as well as complicated skin, pneumonias and other infections requiring hospitalization.
(from: http://www.paratekpharm.com/pt_tet_inhib.html)
Moxifloxacin vs. ciprofloxacin for *S. pneumoniae*...

A. Lismond et al., unpublished
Challenges of efflux in antibacterial chemotherapy

• the way we treat patients:
  – and what about efflux inhibitors?

Thesis:

This will be very difficult because of
• complete changes in the basic rules of drug-design (no link to specific chemical structure) and (porbable) multiplicity of ligand-recognition sites in transporters;
• potential for unanticipated toxicities (related to unknown functions of eucaryotic homologues of the target efflux transporter(s))
Challenges of efflux in antibacterial chemotherapy

- the way we treat patients:
  - is efflux important in pharmacokinetics/drug interactions?

**Thesis:**

Many procaryotic transporters have eucaryotic homologues, and antibiotics are often (for that reason or another …) substrate to them… (sometimes in an unanticipated fashion)
Efflux is widespread because it is a common mechanism of protection of both procaryotic and eucaryotic cells against membrane-permeant toxins... and antibiotics are simply opportunistic substrates (in both types of cells !) ...

Challenges in Chemotherapy:

a new approach (and new problems) in pharmacokinetics (and the potential modulation of antibiotic intracellular activity)

Modulation of cellular pharmacokinetics and intracellular activity: the case of daptomycin

- very bactericidal towards Gram (+) organisms through membrane destabilization (no need of proteinaceous receptor!)
- BUT intrinsically inactive against Gram(-) due to LPS protection
- spare mammalian cells because they lack phosphatidylglycerol (critical for binding to Gram(+) membranes)
- got a fast track registration in the US because of activity against vancomycin-resistant enterococci (VRE)
Models of intracellular infection

*L. monocytogenes*  
*S. aureus*

- Cytosol
- Phagolysosomes
Intraphagoytic S. aureus
A simple scheme ...

metabolism

D'

binding

D

cooperation with host defenses

efflux

D

influx

accumulation and bioavailability

physico-chemical conditions

bacterial responsiveness

Modulation of cellular pharmacokinetics and intracellular activity: the case of daptomycin

Daptomycin activity against intracellular *S. aureus* (THP-1 macrophages)

Lemaire et al., Antimicrob Agents Chemother. 2007 Jun 4; [Epub ahead of print]
Modulation of cellular pharmacokinetics and intracellular activity: the case of daptomycin

Daptomycin activity against intracellular S. aureus (THP-1 macrophages)

Lemaire et al., Antimicrob Agents Chemother. 2007 Jun 4; [Epub ahead of print]
Modulation of cellular pharmacokinetics and intracellular activity: the case of daptomycin

Daptomycin activity against intracellular S. aureus (THP-1 macrophages)

Lemaire et al., Antimicrob Agents Chemother. 2007 Jun 4; [Epub ahead of print]
Modulation of cellular pharmacokinetics and intracellular activity: the case of daptomycin

Daptomycin activity against intracellular S. aureus (THP-1 macrophages)

Lemaire et al., Antimicrob Agents Chemother. 2007 Jun 4; [Epub ahead of print]
Modulation of cellular pharmacokinetics and intracellular activity: 
the case of daptomycin

Lemaire et al., Antimicrob Agents Chemother. 2007 Jun 4; [Epub ahead of print]
Challenges in pharmacokinetics

• efflux will tend to make intracellular activities suboptimal (fluoroquinolones, macrolides, daptomycin…)

• but will it not make antibiotics more toxic?

• and, incidentally, how can we explain/avoid recognition by eucaryotic efflux pumps?

(quiz: daptomycin has a log P of -4.07 and a log D of -9.6 at pH 7… How can it be a substrate of the P-gp?)
Modeling of P-glycoprotein 3d structure

Challenges of efflux in antibacterial chemotherapy

- **recognizing its existence:** is it a major and general means of resistance?
- **its role:** does it need to change our vision on (and decisions about) existing antibiotics?
- **the way we find it:** how can we detect it (and do we need to do this?)
- **the way we treat patients:**
  - can we make non-effluxed drugs?
  - and what about efflux inhibitors?
  - is efflux important in pharmacokinetics/drug interactions?
Let us believe in pumps…
(each has its own set of challenges)

the fire pump…
(save your life)

the gas pump…
(keeps you moving)

the pump for everything …
(white product)

the little amateur one…
but with great expectations …
Let us also believe in pumps operators…
(each met her/his own set of challenges)

- Cristina Seral
- Jean-Michel Michot
- Nancy Caceres
- Ann Lismond
- Sylviane Carbonnelle

- Françoise van Bambeke
- Martine Prevost (ULB)
- Béatrice Marquez

- Narcisa Mesaros
- Laëtitia Avrain
- Youri Glupczynski

- Sandrine Lemaire
- S. Vandevuer
And thank your for the invitation in Bremen ...