Antibiotic transporters in *Pseudomonas aeruginosa* and *Streptococcus pneumoniae*: impact on resistance and clinical significance

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462nd WE Heraeus Seminar
Jacobs University Bremen, 4. - 10. July 2010

Transport across membranes:
Multiple drug resistance, mechanisms and new tools
You said "Impact"?

impact [ɪmˈpækt] noun
the force with which one thing hits another or with which two things hit each other, or (figurative) a powerful effect that something, esp. something new, has on a situation or person

(figurative) The anti-smoking campaign had had/made quite an impact on young people. [U]
(figurative) The new proposals were intended to soften the impact of the reformed tax system. [U]
What is being communicated to the public?

Bad Bugs, No Drugs... 10 New Antibiotics by 2020

IDSA Testimony on Antibiotic Resistance, delivered before the House Energy and Commerce Health Subcommittee (pdf) June 9, 2010
Brad Spellberg, MD, FIDSA, presented IDSA's statement at a hearing on "Promoting the Development of Antibiotics and Ensuring Judicious Use in Humans." FDA's and other statements and the hearing video are located here.

The 10 x '20 Initiative Inaugural Statement (pdf) April 2010
IDSA and other organizations address the dry antibiotic pipeline and call for 10 new antibiotics by 2020.

10 x '20 Endorsing Organizations (pdf) June 29, 2010
What are (some) companies thinking?

- **Antibiotic Market**
  - With annual sales of over $26 billion, antibiotics represent one of the largest therapeutic categories from a revenue perspective.

- **What should we do?**
  - We believe that the growing problem of drug-resistant bacteria will continue to drive growth in new and expanding market opportunities. While much attention has been focused on resistance in Gram-positive pathogens such as methicillin-resistant *Staphylococcus aureus* (MRSA), increasing antibiotic resistance in Gram-negative organisms such as *Pseudomonas aeruginosa* represents a significant threat, with far fewer treatment options available.
But at the same time …

- Public authorities launch campaigns for reducing antibiotic use…

**RESEARCH LETTER**

**Association Between Antibiotic Sales and Public Campaigns for Their Appropriate Use**

JAMA, November 24, 2004—Vol 292, No. 20  **2469**

![Graph showing changes in antibiotic sales over months and campaigns](image)

Residual seasonal autoregressive terms: lag period, 12 months; estimated coefficient: 0.83 [SE, 0.06]; constant: 7.459075 (SD, 431387) defined daily doses/mo. The P values are indicated for the months and campaigns for which the changes were statistically significant.
And "Big Pharma" is leaving the antibiotic area…

- Roche, Bayer, E. Lilly, Sanofi-Aventis … Why ?
  - difficulties in finding truly novel molecules …
  - too low ROI because antibiotics are (i) cheap; (ii) used only for short periods of time… (10 days average)
  - regulatory hurdles for novel compounds (ceftobiprole, oritavancin, iclaprim, telavancin [so far in EU], faropenem) making development uncertain
  - safety issues (over)emphasized for (some) existing compounds by fear of overuse (telithromycin, moxifloxacin) and/or because of inappropriate initial positioning (trovafloxacin)
  - restricted use of novel compounds in several countries (daptomycin, tigecycline) and/or difficulties in positioning when facing generic (or soon generic) equivalents (doripenem)
And "Big Pharma" is leaving the antibiotic area…

- Roche, Bayer, E. Lilly, Sanofi-Aventis …
- too low ROI because antibiotics (i) are cheap; (ii) are used only for short periods of time
- regulatory hurdles for novel compounds [ceftobiprole, oritavancin iclaprim, telavancin, telavancin], [so far in EU], faropenem) making development uncertain
- safety issues (over) emphasized for (some) existing compounds by fear of over usage [telithromycin, moxifloxacin] and/or because of inappropriate initial positioning [trotavfloxacin]
- restricted use of novel compounds in several countries [daptomycin, tigecycline] and/or difficulties in positioning when facing generic (or soon generic) equivalents (doripenem)
A simple price comparison…

• **Treating a community-acquired pneumonia**
  – 5-30 % mortality if left untreated
  – almost 100 % chances of success if appropriate antibiotic
  – average price in Europe for a full treatment (based on guidelines):
    • from 7.88 € (generic amoxicillin, low dose, 7 days)
    • to **127.5 €** for levofloxacin (non-generic, high dose, 10 days)

• **Treating cancer with antibody** (bevacizumab as an example)
  – mean survival: 20.3 vs. 15.6 mo (placebo)
  – response rate: 45 vs. 35 % (placebo)
  – average price for a 1 year treatment (based on official indications for metastatic colorectal-cancer):
    • **63,000 US$** (US prices)
Challenges in the development of an antibiotic acting against resistant strains

- defining the indications for which it must be developed
  - often "niche" or "semi-niche" indications
- defining the level of acceptable risk to the patient
  - stay away from indications where safer compounds are (still) available
- finding it:
  - solving the discovery bottlenecks
- developing and selling it:
  - chemical development
  - preclinical development
  - clinical development
  - pricing …
Is efflux an important mechanism of resistance?

No. of publications in PubMed with keywords: antibiotic AND (efflux OR transporter)
Historical landmarks …

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

Successive description of efflux-mediated resistance for major antibiotics.
But is efflux really important?

- Efflux has long been taken lightly in clinical practice …
  - because it most often causes only low levels of resistance, which have long been considered as "clinically insignificant" …

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

Things are changing: the MPC concept...

"Classic" bactericidal effect

MIC<sub>99</sub> = 0.8

poorly sensitive organisms...

Elimination of resistant organisms

MPC<sub>10</sub> = 9

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

Concentration which will inhibit the majority of the organisms

**MIC\textsubscript{99} = 0.8**

Concentration needed to prevent the selection of resistant organisms

**MPC\textsubscript{10} = 9**

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Dong et al; AAC 43:1756-1758

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Surviving bacteria

Concentration

9th July 2010  462nd WE Heraeus Seminar, Jacobs University, Bremen
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) …
Things are changing: the new breakpoints of EUCAST ...
Before EUCAST …

<table>
<thead>
<tr>
<th>cefotaxime vs. <em>E.coli</em></th>
<th>S&lt; / R</th>
</tr>
</thead>
<tbody>
<tr>
<td>BSAC United Kingdom</td>
<td>2 / &gt;4</td>
</tr>
<tr>
<td>CA-SFM France</td>
<td>4 / &gt;32</td>
</tr>
<tr>
<td>CRG The Netherlands</td>
<td>4 / &gt;16</td>
</tr>
<tr>
<td>DIN Germany</td>
<td>2 / &gt;16</td>
</tr>
<tr>
<td>NWGA Norway</td>
<td>1 / &gt;32</td>
</tr>
<tr>
<td>SRGA Sweden</td>
<td>0.5 / &gt;2</td>
</tr>
</tbody>
</table>

Yet, these breakpoints were used everyday by clinical microbiology laboratories to advise clinicians about which antibiotic(s) they could successfully use against the bacteria they were supposed to fight …
A simple decision ...

<table>
<thead>
<tr>
<th>cefotaxime vs. <em>E.coli</em></th>
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<tr>
<td>NCCLS U.S.A.</td>
<td>8 / &gt;64</td>
</tr>
</tbody>
</table>

Would this not be a smart decision?
A simple decision …

The US clinician can treat all patients!

<table>
<thead>
<tr>
<th>NCCLS</th>
<th>U.S.A.</th>
<th>8 / &gt;64</th>
</tr>
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</table>
EUCAST breakpoints in a nutshell

- based on PK/PD considerations with efficacy in the clinical set-up as the first and most important element of decision
- most often considerably lower than all former (and many current) NCCLS (presently CLSI) breakpoints
- put many isolates with low "resistance mechanisms" in the intermediate or resistant category

more at http://www.eucast.org
An example for *P. aeruginosa* in an Academic Hospital
(Leuven, Belgium)

![Graph showing antibiotic susceptibility levels](image-url)

- **Limit of PK/PD usefulness of levofloxacin**
- **NCCLS levofloxacin R breakpoint**
- **Efflux and first mutants, reported as "S" or "I"**

**J. van Eldere, 2003**
An example for *P. aeruginosa* in an Academic Hospital (Leuven, Belgium)

![Graph showing antimicrobial susceptibility](chart)

- Limit of PK/PD usefulness of levofloxacin
- EUCAST R breakpoint
- All these will now be reported as resistant

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J. van Eldere, 2003
Application for Pseudomonas…

- piperacillin/tazobactam
  - MIC<sub>50</sub> bkpt
  - 1997: 32 (no EUCAST bkpt yet defined)
  - 2000: 16
  - 2005: 8

- ceftazidime
  - MIC<sub>50</sub> %< bkpt
  - 1997: 16 (38)
  - 2000: 8 (53)
  - 2005: 8 (59)

- meropenem
  - MIC<sub>50</sub> %< bkpt
  - 1997: 2 (82)
  - 2000: 1 (76)
  - 2005: 0.5 (78)

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)

- high risk zone (above the EUCASTR breakpoint)
- high risk zone (above the EUCAST R breakpoint)
Application for Pseudomonas...

but this is also a risky zone…
(clinical outcome uncertain)

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)
Study #1: *Pseudomonas* in Brussels in 2010

- Mickaël Riou, Sylviane Carbonnelle, Laëtitia Avrain, Narcisa Mesaros, Qing Tan, Françoise Van Bambeke, Youri Glupczynski
- Jean-Paul Pirnay, Daniel De Vos
- Anne Simon, Denis Piérard, Frédérique Jabobs, Anne Dediste

- Unité de pharmacologie cellulaire et moléculaire, Université catholique de Louvain, Bruxelles
- Coris BioConcept, Gembloux
- Laboratory for Molecular & Cellular Technology, Queen Astrid Military Hospital, Neder-over-Hembeek
- Department of Molecular and Cellular Interactions, Vrije Universiteit Brussel, Brussels;
- Laboratoire de microbiologie, Cliniques universitaires Saint-Luc, Brussels; Laboratorium voor microbiologie, Universitair Ziekenhuis Brussel, Brussels, Service d'infectiologie, Hôpital Erasme, Brussels; Laboratoire de microbiologie, Centre hospitalo-universitaire Saint-Pierre, Brussels; Laboratoire de microbiologie, Cliniques universitaires UCL de Mont-Godinne, Yvoir Belgium.

Riou et al., submitted
What was the problem?

*Pseudomonas aeruginosa: resistance and therapeutic options at the turn of the new millennium*

N. Mesaros1, P. Nordmann2, P. Plésiat3, M. Roussel-Delvallez4, J. Van Eldere5, Y. Glupczynski6, Y. Van Laethem7, F. Jacobs8, P. Lebecque9, A. Malfroot10, P. M. Tulkens1 and F. Van Bambeke1

**ABSTRACT (summarized)**

*Pseudomonas aeruginosa* is a major cause of nosocomial infections.

It resists to many antibiotics, either intrinsically (because of constitutive expression of β-lactamases and efflux pumps, combined with low permeability of the outer-membrane) or following acquisition of resistance genes (e.g., genes for β-lactamases, or enzymes inactivating aminoglycosides or modifying their target), over-expression of efflux pumps, decreased expression of porins, or mutations in quinolone targets.

Susceptibility testing is therefore crucial in clinical practice.

Empirical treatment usually involves combination therapy, selected on the basis of known local epidemiology.

Innovative therapeutic options for the future remain scarce.

Accepted: 24 November 2006

*Clin Microbiol Infect* 2007; 13: 560–578
Epidemiological study

Impact of therapy on the development of in vitro antimicrobial resistance in *Pseudomonas aeruginosa* strains isolated from lower respiratory tract of Intensive Care Units (ICU) patients with nosocomial pneumonia

Supported by the
- "Région Bruxelloise/Brusselse Gewest" (Research in Brussels)
- FNRS (post-doctoral fellowships)
- FRSM
What did we do?

Initial collection
- 144 patients
- 233 isolates

Screening for confirmed VAP / HCAP

104 patients
- 199 isolates

35 patients with D0 isolate(s) only
- 38 isolates

69 patients with multiple successive samples
- 161 isolates

Clonality analysis

Non clonal isolates (10) (only initial isolate kept)

- Erasme
- UZ Brussel
- St-Luc
- St Pierre

- UCL
- Queen Astrid Military Hospital

Riou et al., submitted
What did we do?

69 patients with multiple successive samples
161 isolates

clonality analysis

Non clonal isolates (10) (only initial isolate kept)

59 patients
62 clonal isolate pairs

62 day 0 (D0)

62 last day (DL)

D0 isolates (110)

59 patients
62 clonal isolate pairs

62 day 0 (D0)

pairs (D0-DL) 2 x 62

• Queen Astrid Military Hospital

Riou et al., submitted
What is the situation at day 0?

MIC (mg/L: 0.0156 to 512 mg/L)

- - - - - EUCAST bkpt > R
-------- CLSI bkpt ≥ R

Riou et al., submitted
What is the situation at day 0?

- **Gentamicin**
  - EUCAST bkpt > R
  - CLSI bkpt ≥ R

- **Piperacillin**
  - EUCAST bkpt > R
  - CLSI bkpt ≥ R

- **Ticarcillin**
  - EUCAST bkpt > R
  - CLSI bkpt ≥ R

- **Aztreonam**
  - EUCAST bkpt > R
  - CLSI bkpt ≥ R

- **Polistin**
  - EUCAST bkpt > R
  - CLSI bkpt ≥ R

Riou et al., submitted
What are the susceptibilities at day 0 if you have received (or not) the same antibiotic up to 1 month before?

- individual values with geometric mean (95 % CI)
- S (lowest line) and R (highest line) EUCAST breakpoints

* p < 0.05 by unpaired t-test (two-tailed) and Mann-Whitney non-parametric test

Riou et al., submitted
What happens during treatment?

- 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)
Are the antibiotics the cause of the problem?

<table>
<thead>
<tr>
<th>antibiotic</th>
<th>use (%)</th>
<th>non susceptible isolates according to</th>
<th>loss of susceptibility (%) during treatment</th>
<th>and correlation with antibiotic use</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>EUCAST (% I / R)</td>
<td>CLSI (% I / R)</td>
<td></td>
</tr>
<tr>
<td>D0</td>
<td>DL</td>
<td>D0</td>
<td>DL</td>
<td>EUCAST</td>
</tr>
<tr>
<td>AMK</td>
<td>22.0</td>
<td>1.6 / 11.3</td>
<td>11.3 / 16.1</td>
<td>0.0 / 11.3</td>
</tr>
<tr>
<td>CIP</td>
<td>8.3</td>
<td>4.8 / 25.8</td>
<td>4.8 / 35.5</td>
<td>3.2 / 22.6</td>
</tr>
<tr>
<td>MEM</td>
<td>21.2</td>
<td>12.9 / 22.6</td>
<td>14.5 / 35.5</td>
<td>1.6 / 22.6</td>
</tr>
<tr>
<td>TZP</td>
<td>23.5</td>
<td>33.9 &lt;sup&gt;d&lt;/sup&gt;</td>
<td>53.2 &lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.0 / 17.7</td>
</tr>
<tr>
<td>FEP</td>
<td>22.0</td>
<td>40.3 &lt;sup&gt;d&lt;/sup&gt;</td>
<td>53.2 &lt;sup&gt;d&lt;/sup&gt;</td>
<td>12.9 / 27.4</td>
</tr>
<tr>
<td>CAZ</td>
<td>3.0</td>
<td>35.5 &lt;sup&gt;d&lt;/sup&gt;</td>
<td>46.8 &lt;sup&gt;d&lt;/sup&gt;</td>
<td>8.1 / 27.4</td>
</tr>
</tbody>
</table>

<sup>a</sup> **red bold**: resistance in > 25 % of all isolates
<sup>b</sup> % of isolates moving from S to I or R between day 0 and day ≥ 3
<sup>c</sup> non parametric correlation (Spearman rank) between the % of use of each antibiotic (% of all antibiotic prescriptions) in the whole population (AMK, 24.0; CIP, 9.6; MEM, 20.2; FEP, 15.4; CAZ, 3.8) and the increase in % of isolates with change in susceptibility (moving from S to I, I to R, or S to R) for the corresponding antibiotic
<sup>d</sup> no intermediate category for EUCAST

Riou et al., submitted
But what happened with the patients?

<table>
<thead>
<tr>
<th>Clinical outcome</th>
<th>alive</th>
<th>death from</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>41</td>
<td>9</td>
</tr>
<tr>
<td>no. of patients</td>
<td>41</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>other cause</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>9</td>
</tr>
</tbody>
</table>

assessed after 90 days following the collection date of the first isolate except for 2 patients (alive) for whom the observation period was extended to 202 and 213 days.
But what happened to the bacteria?

• "classical" resistance
• efflux-mediated resistance
Classical resistance...

- Antibiogram (with interpretation) at high and low density inocula
- Direct genomic determination for suspected mechanisms (enzymes, porins ...)
  - Multiple mechanisms ...

Hard work still in progress ...
Efflux and clinical resistance in *Pseudomonas aeruginosa* at the onset of treatment (day 0)

**MICs vs EUCAST breakpoints for 109 *P. aeruginosa* without or with efflux mechanisms, isolated from ICU patients (VAP)**
Efflux pumps overexpression *

<table>
<thead>
<tr>
<th>Type of PCR</th>
<th>Genetic status</th>
<th>Day 0 (% of isolates)</th>
<th>Day X (% of isolates)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Real time PCR</strong> (constitutive genes)</td>
<td>MexA- / MexX-</td>
<td>66.13</td>
<td>38.71</td>
</tr>
<tr>
<td></td>
<td>MexA+ / MexX-</td>
<td>19.90</td>
<td>22.58</td>
</tr>
<tr>
<td></td>
<td>MexA- / MexX+</td>
<td>11.29</td>
<td>20.97</td>
</tr>
<tr>
<td></td>
<td>MexA+ / MexX+</td>
<td>9.68</td>
<td>17.74</td>
</tr>
<tr>
<td><strong>Classical PCR</strong> (inductive genes)</td>
<td>MexC- / MexE-</td>
<td>90.50</td>
<td>87.00</td>
</tr>
<tr>
<td></td>
<td>MexC+ / MexE-</td>
<td>6.50</td>
<td>11.00</td>
</tr>
<tr>
<td></td>
<td>MexC- / MexE+</td>
<td>3.00</td>
<td>6.50</td>
</tr>
<tr>
<td></td>
<td>MexC+ / MexE+</td>
<td>0.00</td>
<td>5.00</td>
</tr>
</tbody>
</table>

* Gene expression evaluated by Real Time PCR (mex Q-Test Kit, Coris BioConcept) for mexA (constitutively expressed) and mexX (inducible with low expression level in WT strains), and by PCR on cDNA for mexC and mexE (repressed in WT strains).

Riou et al., ECCMID 2010
Efflux selection during treatment

Prevalence of MexA and MexX overexpressers in 62 phylogenetically-related pairs of *P. aeruginosa* isolated from ICU patients (VAP)

Riou et al., ECCMID 2010
Efflux selection during treatment

Antipseudomonal antibiotics received by the patients during treatment

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>no. patients</th>
<th>69% combinations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Piperacillin-tazobactam</td>
<td>26</td>
<td></td>
</tr>
<tr>
<td>Amikacin</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td>Meropenem</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Cefepime</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>6</td>
<td></td>
</tr>
</tbody>
</table>

Global influence of treatment

Number of strains detected at day 0 and day X

Riou et al., ECCMID 2010
What happens if you overexpress MexA and/or MexX?

\[ \square \text{mexA} \quad \blacksquare \text{mexX} \]

You increase your MIC by 2 to 5 dilutions… and you cross the S/R breakpoint…

Mesaros et al., JAC (2007) 59:378-386
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 need to be combined (E-test with mRNA detection)
  – Proteomic tests (using antibody-based detection techniques) could be added also.

http://www.corisbio.com
Study #2: *Streptococcus pneumoniae* in Belgium in 2010

- Ann Lismond, Farid El Garch, Sibille Delvigne, Sylviane Carbonnelle, Françoise Van Bambeke
- Mark Garvey, Laura Piddock, Jean-Paul Pirnay, Daniel De Vos
- Frank Verschuren, Fréderique Jacobs, Denis Pierard, Paul Jordens

- Unité de pharmacologie cellulaire et moléculaire, Université catholique de Louvain, Bruxelles
- Antimicrobial Research Group, University of Birmingham, Birmingham
What do we have for fluoroquinolones (CAP patients) ?

A. Cumulative MIC distribution for 134 isolates of *S. pneumoniae* towards levofloxacin, moxifloxacin, garenoxacin and gemifloxacin in the absence (solid lines) or presence (dotted lines) of 10mg/L reserpine.

Lismond *et al.* ECCMID 2009
And with "reporter" fluoroquinolones" (COPD patients; n=107) ?

Effect of reserpine on MIC distributions of ciprofloxacin and norfloxacin

Lismond et al. ECCMID 2010
Does this represent efflux?

Suspected efflux based on phenotypic analysis

- Norflo: 42%
- Cipro + Norflo: 34%
- Aucun: 18%
- Cipro: 6%

Lismond et al. ECCMID 2010
Does this represent efflux?

Suspected efflux based on genotypic analysis

Lismond et al. ECCMID 2010
Can \textit{pmrA} and \textit{patA/patB} be induced?

Induction of \textit{pmrA}, \textit{patA}, and \textit{patB} expression in \textit{S. pneumoniae} exposed for 4 h to half MIC of various fluoroquinolones. Data are presented as the ratios of expression measured in induced and non-induced conditions. Values are the mean $\pm$ SEM of duplicates from 2 independent experiments. Statistical analysis: *: $p < 0.05$ (one-way ANOVA with Dunnett’s post-hoc test for comparison with non-induced condition).

El Garch \textit{et al.}, JAC, in press
Is patA/patB induced overexpression fast ... and reversible?

- Kinetics of induction and desinduction of patA and patB expression by ciprofloxacin and moxifloxacin in S. pneumoniae ATCC49619
- Exposure to half MIC of ciprofloxacin or moxifloxacin during 6 h.
- For reversion, bacteria induced for 4 h were harvested and regrown in broth without antibiotic for 5 h.

El Garch et al., JAC, in press
What should we conclude?

- Efflux is a reality and cause detectable, clinically-meaningful resistance … which will now be reported more and more by clinical microbiologists (study #1 - *Pseudomonas*)
- Antibiotics induce efflux, even those which are not (apparent) substrate… (study #2 – *Pneumococci*)
- This affects several classes of antibiotics (cross-resistance)

- So, the impact is at two levels:
  - decreased choice for the clinician
    - novel antibiotics are a must …
  - new challenge for the drug designer
    - novel antibiotics should not only be non-substrate but should also not induce efflux … of other drugs…)

9th July 2010  462nd WE Heraeus Seminar, Jacobs University, Bremen
And thank your for the invitation in Bremen ...
And here is (part of) the "pump team"
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