The Major Mechanisms of Resistance to Aminoglycosides, Macrolides, Fluoroquinolones

Paul M. Tulkens, MD, PhD

Cellular and Molecular Pharmacology
Louvain Drug Research Institute
Université catholique de Louvain
Brussels, Belgium

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Disclosures and slides availability

• Research grants
  – Theravance, Astellas, Targanta, Cerexa/Forest, AstraZeneca, Bayer, GSK, Trius, Rib-X, Eumedica
  – Belgian Science Foundation (F.R.S.-FNRS), Ministry of Health (SPF), and Walloon and Brussels Regions

• Speaking fees
  – Bayer, GSK, Sanofi, Johnson & Johnson, OM-Pharma, Cubist

• Decision-making and consultation bodies
  – General Assembly and steering committee (2008-2010) of EUCAST
  – European Medicines Agency (external expert)
  – US National Institutes of Health (grant reviewer)

Slides: http://www.facm.ucl.ac.be ➔ Lectures
The aminoglycosides in short

- The first class of wide-spectrum antibiotics
  (as the result of a first systematic screening of natural sources)

- Concentration-dependent and highly bactericidal

- Active of most aerobic Gram-negative bacteria including *P. aeruginosa*

- For some of them, also active against *Mycobacterium tuberculosis* and other *Mycobacteria*

- Synergy with cell-wall acting agents

- Resistance variable between regions and settings but usually low ... because of limited use
From the point of view of human benefit, never was a Nobel prize so justifiably awarded as was the award to Selman Waksman for the discovery of streptomycin and other antibiotics produced from Streptomyces spp. Waksman and his talented team (many of whom went on to make important antibiotic discoveries in their own right) developed the concept of systematic screening of microbial culture products for biological activity, a technology which has provided the foundation of the antibiotic industry, and for this alone his name should rank high in any pantheon of microbiology.

J. Davies: In Praise of Antibiotics, ASM News
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Concentration-dependence

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Resistance of *P. aeruginosa* to antibiotics in ICU (VAP patients; Belgian hospitals; 2007-2009)

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

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

Graphs showing cumulative percentage of resistance for each antibiotic:
- **Amikacin**
- **Ciprofloxacin**
- **Meropenem**
- **Piperacillin / Tazobactam**
- **Cefepime**
- **Ceftazidime**
Aminoglycosides: main mechanisms of resistance

• Intrinsic resistance
  – All anaerobic bacteria (no accumulation in bacteria)
  – Enterococci (facultative anaerobics)
  – Bacteria with mutations at 16S rRNA (resistant *M. tuberculosis* (includ. point mutations: *M. abscessens*, *M. chloanae*)

• Acquired (or resulting from overexpression) resistance
  – Reduced entry and efflux
  – Enzymatic modification
  – Methylation of target
  – Small colony variants
  – Biofilms
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Aminoglycosides: efflux

- Mostly related to the presence tripartite pumps (MexXY-OprM in *P. aeruginosa*; AcrA/B-TolC in *Enterobacteriaceae*; AmrAB in *Burkholderia*…)

- Recognition may be after association/binding to phospholipids (amphiphilic complex) but may also involve specific recognition sites

- Probably responsible for
  - Decreased susceptibility during treatment and/or exposure to sub-inhibitory concentrations (overexpression)
  - Adaptative resistance (_spike susceptibility after high peak_)

2/12/2014 2014 ISF Stephen F. Lowry Colloquium
Asymmetric trimer model of MexY
(homology modeling on the crystal structure of *E. coli* AcrB)


Prevalence of efflux pumps in 62 pairs of *Pseudomonas aeruginosa* collected from ICU patients

- The prevalence of *mexA*+ and *mexX*+ was already high in first isolates.
- For all genes tested, the number of isolates with overexpression increased during treatment (P < 0.05, Fisher's Exact Test 2-sided).

Riou et al. 20th ECCMID, 2010, Poster 780
Riou et al., in preparation
Aminoglycosides: enzymatic modification

kanamycin B

2"-adenylation
2"-phosphorylation
3'-phosphorylation
2'-acetylation
4'-adenylation
6'-acetylation
3-acetylation

gentamicin C1 : R1 = CH3; R2 = CH3

gentamicin C1a: R1 = H; R2 = H

gentamicin C2: R1 = CH3; R2 = H

kanamycin A
Enzymatic modifications: the situation in the late 90’s

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: the family
(based on susceptibility to enzymatic resistance mechanisms)

<table>
<thead>
<tr>
<th>group</th>
<th>typical approved or <em>in development</em> antibiotics</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>most susceptible</td>
</tr>
<tr>
<td>Streptomycins</td>
<td>streptomycin</td>
</tr>
<tr>
<td>Neomycins</td>
<td></td>
</tr>
<tr>
<td>Kanamycins</td>
<td>kanamycin A</td>
</tr>
<tr>
<td></td>
<td>kanamycin B</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Gentamicin</td>
<td>“gentamicin” *</td>
</tr>
<tr>
<td><em>gentamicin B</em></td>
<td></td>
</tr>
<tr>
<td>Sisomicin</td>
<td>sisomicin</td>
</tr>
<tr>
<td>Spectinomycin</td>
<td></td>
</tr>
</tbody>
</table>

* Mixture of gentamicins C1, C1a, C2 and C2a
Plazomycin...

sisomicin

still susceptible to AAC(2′) (but rare enzyme)

plazomicin
Plazomycin...
Aminoglycosides: main mechanisms of resistance

• **Intrinsic resistance**
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• **Acquired (or resulting from overexpression) resistance**
  – Reduced entry and efflux
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  – Small colony variants
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Exogenously acquired 16S rRNA methyltransferase (16S-RMTase) genes responsible for a very high level of resistance against various aminoglycosides have been widely distributed among *Enterobacteriaceae* and glucose-nonfermentative microbes recovered from human and animal.

Methylases may be part of the normal bacterial “equipment”
Methylases may be part of the normal bacterial “equipment”
Macrolides

« OLD » MACROLIDES

14-membered

15-membered

16-membered

<table>
<thead>
<tr>
<th></th>
<th>X</th>
<th>R</th>
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<tbody>
<tr>
<td>Erythromycin</td>
<td>C=O</td>
<td>H</td>
</tr>
<tr>
<td>Roxithromycin</td>
<td>C=N-O-CH₂-O-CH₂-O-CH₃</td>
<td>H</td>
</tr>
<tr>
<td>Clarithromycin</td>
<td>C=O</td>
<td>CH₃</td>
</tr>
<tr>
<td>Erythromycylamine</td>
<td>C-NH₂</td>
<td>H</td>
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</table>

<table>
<thead>
<tr>
<th></th>
<th>R₁</th>
<th>R₂</th>
<th>R₃</th>
<th>R₄</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spiramycin</td>
<td>H</td>
<td>Forosamine</td>
<td>H</td>
<td>H</td>
</tr>
<tr>
<td>Josamycin</td>
<td>COCH₃</td>
<td>H</td>
<td>H</td>
<td>COCH₂CH(CH₃)₂</td>
</tr>
<tr>
<td>Miocamycin</td>
<td>COCH₂CH₃</td>
<td>COCH₃</td>
<td>COCH₃</td>
<td>COCH₂CH₃</td>
</tr>
<tr>
<td>Rokitamycin</td>
<td>H</td>
<td>H</td>
<td>COCH₂CH₃</td>
<td>CO(CH₂)₂CH₃</td>
</tr>
</tbody>
</table>

Macrolides main mechanisms of resistance

• Intrinsic resistance
  – Gram-negative bacteria: impermeability
    (with exceptions and possible modulation by the medium)

• Acquired
  – Efflux
    • msrA in Staphylococci (MSB phenotype)
    • mefA in Streptococci and Enterococci
      (dissociation between clindamycin and macrolides)
  – Target mutations
    • dimethylation of the A2058 residue within a conserved region of domain V of the 23S rRNA
      (several genes and cross-resistance with clindamycin)
  – Drug inactivation (phosphotransferases, esterases…)

Most frequent in Europe
Can macrolides be active against *P. aeruginosa*?

Increased Susceptibility of *Pseudomonas aeruginosa* to Macrolides and Ketolides in Eukaryotic Cell Culture Media and Biological Fluids Due to Decreased Expression of *oprM* and Increased Outer-Membrane Permeability

Julien M. Buyck,^1^ Patrick Pléiat,^2^ H. Traore,^3^ F. Vanderbiyt,^3^ Paul M. Tulkens,^1^ and Françoise Van Bambeke^1^

^1^Pharmacologie cellulaire et moléculaire, Louvain Drug Research Institute, Université catholique de Louvain, and ^2^Laboratoires SMB, Brussels, Belgium; and ^3^Laboratoire de Bactériologie, Hôpital Jean Minjoz, Besançon, France

*Clinical Infectious Diseases* (2012) 55:534-542
Can macrolides be active against *P. aeruginosa*?

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“Old” macrolides are no longer usable for “common” respiratory infections due to *S. pneumoniae*

Community-acquired pneumonia 2006-2009  

Chronic obstructive pulmonary disease 2010-2013  
(bacterial exacerbations)  
Macrolide resistance: mechanisms...

Most of the resistant isolates in Europe are “high level” and “methylase” positive

But clinical isolates may have both mechanisms… or additional ones

A possible future for macrolides…

KETOLIDES

11-N-ketolides

fluoroketolides

6-O-ketolides

bridged bicyclic ketolides

Solithromycin

Cethromycin

Modithromycin
Solithromycin and resistance to “old” macrolides …

S. pneumoniae isolates from Belgium and Germany (n=741)

submitted
Fluoroquinolones

• The first major class of antibiotics of synthetic origin
  (no obvious equivalent in natural products)
  → resistance was though to be unlikely…

• But…
  – spontaneously occurring mutations in chromosomal genes that alter the target
    enzymes (DNA gyrase and topoisomerase IV)
    → high frequency: $10^{-7} / 10^{-8}$ !
  – Efflux-mediated reduction of intrabacterial concentration in both Gram-positive and
    Gram-negative bacteria, even if not pre-exposed to the same molecule
    → favours the selection of resistant mutants
  – Acquisition of plasmid-encoded $qnr$ genes protecting DNA gyrase and
    topoisomerase IV from quinolone action
    → spreading…
  – modification of a plasmid-encoded $aminoglycoside$ acetylating enzyme ($AAC(6')$-Ib-
    cr) that cetylates the C7 aminofunction of piperazinyl-substituted fluoroquinolones
    (ciprofloxacin, norfloxacin).
Fluoroquinolones: prevalence of resistance

Figure 1. Current estimates of resistance to ciprofloxacin among isolates recovered from hospitals in the United States [2–5]. ESBL*, extended-spectrum β-lactamase producing; MRSA, methicillin (or oxacillin)–resistant *Staphylococcus aureus*; VRE, vancomycin-resistant enterococci.

Fluoroquinolones: Mutant Prevention Concentration (MPC)

$\text{MIC}_{99} = 0.8 \text{ mg/L}$ (in this example)

"Classic" bactericidal effect

Elimination of resistant organisms...

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

Dong et al: AAC 1999; 43:1756-1758
Fluoroquinolones: Mutant Prevention Concentration (MPC)

Concentration that inhibits the majority of the organisms

Concentration needed to prevent the selection of resistant organisms (about 10 x the MIC)

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

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

Dong et al; AAC 43:1756-1758
Fluoroquinolones: a high peak may be necessary

Shape does matter: short high-concentration exposure minimizes resistance emergence for fluoroquinolones in *Pseudomonas aeruginosa*

Vanessa E. Rees¹, Jürgen B. Bulitta¹,2†, Roger L. Nation¹, Brian T. Tsuji², Fritz Sörgel³,⁴ and Cornelia B. Landersdorfer¹,2⁎†

¹Drug Delivery, Disposition and Dynamics, Monash Institute of Pharmaceutical Sciences, Monash University (Parkville Campus), Parkville, Victoria 3052, Australia; ²School of Pharmacy and Pharmaceutical Sciences, University at Buffalo, State University of New York, Buffalo, New York, USA; ³IBMP—Institute for Biomedical and Pharmaceutical Research, Paul-Ehrlich-Str. 19, Nürnberg-Heroldsberg, Germany; ⁴Institute of Pharmacology, Faculty of Medicine, University of Duisburg-Essen, Essen, Germany
Fluoroquinolones: a high peak may be necessary

• Delivering the same $f\text{AUC}/\text{MIC}$ over short durations of exposure (i.e. 1, 4 or 10h) achieved more rapid killing with no or very limited emergence of resistance, whereas longer durations of exposure over 16 and 24h led to a dramatic ($5 \log_{10}$) increase in the concentration of resistant bacteria.

• Clinical ciprofloxacin regimens with high intensity, short-exposure durations may provide extensive and rapid bacterial killing with no or limited resistance.
**Fluoroquinolones: dissociated resistance and low MICs**

Fig. 4. Cross-resistance and dissociated resistance in quinolones. $Q_A$ and $Q_B$ illustrate a situation of cross-resistance: although the initial susceptibility of the strain may be different for molecules A and B, mutations in the target enzymes lead to similar changes in the susceptibility to both drugs. $Q_C$ illustrates a situation of dissociated resistance: the susceptibility to molecule C does not change in spite of the acquisition of a first mutation, and will increase only upon acquisition of a second mutation.


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**FIG. 2.** Comparative susceptibilities of various *S. aureus* isolates to moxifloxacin (circles) or delafloxacin (squares). MICs were measured at pH 7.4, and strains are ranked based on their susceptibility to moxifloxacin. Resistance phenotypes and/or strain source are designated by lowercase letters along the x axis: a, animal MRSA; c, CA-MRSA; e, efflux (NorA); h, HA-MRSA; l, linezolid-resistant; m, characterized mutations in fluoroquinolone targets; s, MSSA.
Fluoroquinolones: preexisting efflux
(in *S. pneumoniae* [Belgian CAP isolates])

---

**Ciprofloxacin**
- Concentrations: 3.9 × 10^{-3} - 7.8 × 10^{-3} mg/L
- % isolates: 3.9 × 10^{-3} - 7.8 × 10^{-3} mg/L
- Control and + reserpine graphs show different isolates at various mg/L levels.
- Never used in Europe.

**Moxifloxacin**
- Concentrations: 3.9 × 10^{-3} - 7.8 × 10^{-3} mg/L
- % isolates: 3.9 × 10^{-3} - 7.8 × 10^{-3} mg/L
- Control and + reserpine graphs show different isolates at various mg/L levels.
- Not recommended.

**Gemifloxacin**
- Concentrations: 3.9 × 10^{-3} - 7.8 × 10^{-3} mg/L
- % isolates: 3.9 × 10^{-3} - 7.8 × 10^{-3} mg/L
- Control and + reserpine graphs show different isolates at various mg/L levels.

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# Efflux and selection of resistance

## Change in MICs of Levofloxacin in *Pseudomonas aeruginosa* if deleting the efflux pump operons

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<thead>
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<th>Frequency of LVX-resistant mutants</th>
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<tr>
<td>WT</td>
<td>0.25</td>
<td>$2 \times 10^7 - 4 \times 10^7$</td>
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<tr>
<td>$\Delta$ mexAB-oprM</td>
<td>0.015</td>
<td></td>
</tr>
<tr>
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<td></td>
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Lomovskaya *et al.*, AAC (1999: 43:1340-1346)

The MIC falls to low values ...
**Efflux and selection of resistance**

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<tr>
<td>$\Delta$ mexCD-oprJ; $\Delta$ mexEF-oprN</td>
<td>0.25</td>
<td>$2 \times 10^6$</td>
</tr>
<tr>
<td>$\Delta$ mexAB-oprM; $\Delta$ mexCD-oprJ</td>
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<td>$1 \times 10^9$</td>
</tr>
<tr>
<td>$\Delta$ mexAB-oprM; $\Delta$ mexCD-oprJ; $\Delta$ mexEF-oprN</td>
<td>0.015</td>
<td>$&lt;1 \times 10^{11}$</td>
</tr>
</tbody>
</table>

*AND the selection of mutants in FQ target becomes undetectable when ALL pumps are disrupted*

Lomovskaya et al, AAC (1999) 43:1340-1346
Thank you for your attention!

And ask questions