Emerging aminoglycoside resistance among Enterobacteriaceae and non-fermenters

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ESCMID postgraduate course
Al Ain, February 11th, 2012
Outline

- Aminoglycosides
- Mode of action
- Clinical applications
- Resistance mechanism
- Changing pattern and new challenges
The discovery

Streptomycin – 1943
from *Streptomyces griseus*

A drug with activity against Gram positive and negative organisms and effective against *M. tuberculosis*

„It was on October 19, 1943, at about 2:00 in the afternoon, that I realized I had found a new antibiotic”

Schatz, A.: The true story of the discovery of streptomycin

Rutgers University

Albert Schatz
(1922-2005)

Selman Waksman
(1888-1973)

Actinomycin
Clavacin
Streptothrichin
Grisein
Neomycin
Fradacin
Candididin
Candidin
Basic structure

An aminocyclitol ring

- either streptamine or deoxystreptamine
  - all important aminoglycosides

- or stretidine
  - in streptomycine
The aminocyclitol nucleus is connected through glycosidic linkages to various aminosugars → AMINOGLYCOSIDES

Exception – spectinomycin, it is an aminocyclitol not an aminoglycoside
# Groups

*Finch RG et al. Antibiotic and Chemotherapy, Saunders 2010*

## 2-Deoxystreptamine-containing aminoglycosides

<table>
<thead>
<tr>
<th>Gentamicin group</th>
<th>Kanamycin group</th>
<th>Neomycin group</th>
<th>Other aminoglycosides /aminocyclitols</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gentamicin</td>
<td>Amikacin</td>
<td>Neomycin</td>
<td>Astromicin</td>
</tr>
<tr>
<td>Isepamicin</td>
<td>Arbekacin</td>
<td>Paromomycin</td>
<td>Spectinomycin</td>
</tr>
<tr>
<td>Micronomicin</td>
<td>Dibekacin</td>
<td></td>
<td>Streptomycin</td>
</tr>
<tr>
<td>Netilmicin</td>
<td>Kanamycin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sisomicin</td>
<td>Tobramycin</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Chemical features

- Strongly polar
- Positively charged (cationic)
- Highly soluble in water
- Relatively insoluble in lipids
- Active in alkaline, but less active in acidic environment
- Binds well to negatively charged LPS and outer membrane, RNA, DNA
- Poor absorption from the gut
Uptake

**NON-ENERGY DEPENDENT PHASE**

Electrostatic binding to the negatively charged LPS and OM

*This displaces cations from LPS, enhances permeability which increases uptake*

Diffusion through porin channels

**ENERGY DEPENDENT PHASE**

Transport through CM - oxygen-dependent energy generated by the membrane-bound respiratory chain

*Hence anaerobs are intrinsically resistant enterococci have decreased susceptibility Drugs have decreased activity in anaerobic environment*

Subsequent misreading during translation – misfolded proteins incorporating into the CM – loss of membrane integrity

*Highly increased energy dependent uptake*
Mode of action – interference with protein synthesis

50S subunit (5S + 23S RNA + 33 proteins)

30S subunit (16S + 20 or 21 proteins)
Mode of action – interference with protein synthesis

As aminoglycosides bind to the 16S RNA, they interfere with the recognition of the cognate (correct) tRNA and/or with the proofreading.

A noncognate (incorrect) amino acid is incorporated into the peptide.

Chain terminates or incorrect peptide is produced.

Bacteriocidia.
Mode of action – interference with protein synthesis

Inhibits translocation of the peptidyl-tRNA from the A site to the P

Peptide synthesis stops

Bacteriostasis
Bacteriocidal effect

**MODE**

- Interference with protein synthesis
- Secondary effect
  - loss of membrane integrity due to incorporation of altered proteins
  - increased uptake of drug

**CHARACTERISTICS**

- Concentration dependent effect
- Post-antibiotic effect
## Pros and Cons

**ADVANTAGES**
- Rapid bactericidal action
- Relatively low cost
- Chemical stability
- Broad spectrum activity
- No allergic reaction
- Synergistic action with other antibiotics
- Postantibiotic effect

**DISADVANTAGES**
- Inactivity against anaerobes
- Narrow therapeutic index
- Toxicities: nephrotoxicity, ototoxicity
- Lack of oral absorption

*Based on Jana S and Deb JK Appl Microbiol Biotech 2006*
Examples of use

<table>
<thead>
<tr>
<th>AMINOGLYCOSIDE</th>
<th>APPLICATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Streptomycin</td>
<td>tularemia, tuberculosis and plague</td>
</tr>
<tr>
<td>Gentamicin, amikacin, netilmicin</td>
<td>sepsis, pneumonia, meningitis</td>
</tr>
<tr>
<td>Paromomycin</td>
<td>amoebic dysentery</td>
</tr>
<tr>
<td>Spectinomycin</td>
<td>gonorrhoea</td>
</tr>
<tr>
<td>Neomycin</td>
<td>burns, wounds, ulcers, dermatitis</td>
</tr>
</tbody>
</table>
Typical modes of administration

- Single daily dose
- Parenteral
- Nebulised compounds (tobramycin, gentamicin)
- Topical
- Liposome-encapsulated compounds
### Mechanisms of resistance

<table>
<thead>
<tr>
<th>Mechanism</th>
<th>Details</th>
</tr>
</thead>
</table>
| Reduction of intracellular concentration | - changes in OM permeability  
 |  
 |  
 |  
 |  
 | - decreased CM transport  
 |  
 |  
 | - efflux  
 |  
 |  
| Enzymatic modification of the drug | - mutation  
 |  
 |  
 |  
 | - enzymatic modification  
 |  
 |  
| Alteration of the target (ribosome) | - mutation  
 |  
 |  
 |  
 | - enzymatic modification  
 |  
 |  

Usually more decreased susceptibility than real resistance

Porin loss – *P. aeruginosa*

Decreased surface negative charge – *P. aeruginosa*

CM transport - naturally absent in anaerobes
- mutations affecting the membrane coupled respiratory chain resistance
- slow growth
- persistance during therapy
*P. aeruginosa, E. coli, S. aureus*
Mechanisms of resistance – efflux pumps

ATP-DEPENDENT

ATP-Binding Cassette (ABC family)

PROTON ANTIPORTERS

Major Facilitator Superfamily (MFS)
Small Multidrug Resistance family (SMR)
Resistance Nodulation Division family (RND)

Na+/H+ DRUG ANTIPORTER

Multidrug And Toxic compound Extrusion family (MATE)

Often associated with MDR

Constitutive expression usually results in low level resistance

Hyper-expression due to mutation or substrate induction may result clinically significant (multi-drug) resistance
Mechanisms of resistance – modification of the drug

- Aminoglycoside phosphotransferases (APHs)
- Aminoglycoside acetyltransferases (AAHs)
- Aminoglycoside nucleotidyltransferases (ANHs)

The genes often located on plasmids, transposons (together with other R genes)

**AAC(6′)-Ia**

- **Family** (mode of action)
- **Class** – site of modification
- **Type** – resistance phenotype

Same class and type but different genes
# Range of enzyme activity

*Simplified from Finch RG et al. Antibiotic and Chemotherapy, Saunders 2010*

Only strong actions are shown

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Kanamycin A</th>
<th>Neomycin</th>
<th>Amikacin</th>
<th>Tobramycin</th>
<th>Gentamicin</th>
<th>Netilmicin</th>
<th>Sisomicin</th>
</tr>
</thead>
<tbody>
<tr>
<td>APH(3′)-I, II, IV, VII</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>APH(3′)-III &amp; VI</td>
<td></td>
<td></td>
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<tr>
<td>APH(3′)-V</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>APH(2″)</td>
<td></td>
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<tr>
<td>ANT(4′)</td>
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<tr>
<td>ANT(2″)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>AAC(3)-I &amp; VI</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AAC(3)-II</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AAC(3)-III</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AAC(3)-IV</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AAC(2′)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AAC(6′)-I</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AAC(6′)-II</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Narrow

Broad
Distribution of modifying enzymes

1970-1980ies

**USA**  
- **gentamicin** was the most frequently used aminoglycoside  
- ANT(2")-I (Kana, Tobra, Genta)  
- AAC(3)-I (Genta)

**Japan**  
- **amikacin** was preferred  
- ANT(2")-I (Kana, Tobra, Genta)  
- AAC(6’)-I (Amik, Netil, Tobra, Kana, Dibekacin) (but NOT Genta)

*Price, KE et al. 1981 JAC 8(SupplA):89*  
*Shimizu, K et al. 1985 AAC 28:282*  
*Vakulenko SB et al. 2003 CMR 16:430*
Distribution of modifying enzymes

The local intensity and pattern of the use of aminoglycosides affects the local incidence of various enzymes, although the pattern of resistance has been increasingly complex.

<table>
<thead>
<tr>
<th>Group</th>
<th>Gentamicin</th>
<th>Tobramycin</th>
<th>Amikacin</th>
<th>Netilmicin</th>
</tr>
</thead>
<tbody>
<tr>
<td>G group</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>GT group</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>GA group</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>GTA group</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Hospitals using:

- G group: gentamicin
- GT group: gentamicin + tobramycin
- GA group: gentamicin + amikacin
- GTA group: gentamicin + tobramycin + amikacin
Target modification – ribosomal mutations

Mutations affecting the 16S rRNA \((rrs)\) or the ribosomal proteins

Streptomycin resistance in

- *M. tuberculosis*
- enterococci
- *S. aureus*
- *N. gonorrhoeae* (also to spectinomycin)
Several aminoglycoside-producing *Actinomycetes* were known to produce 16S methylases to protect themselves. Any clinical significance?

**2002**

The sequence of a gene (later called *armA*), a 16S methylase found in *C. freundii* isolated in **Poland** was deposited in GenBank (AF550415) co-located on a plasmid also coding for CTX-M3

(Golebiewski, M et al. 2007 AAC 51:3789)

**2003**

**Japan** - an aminoglycoside resistant *P. aeruginosa* producing a 16S methylase (*rmtA*)

High level of resistance to all 4,6 –deoxystreptamines

(Yokoyama K et al. 2003 Lancet 47:1888)

The same gene (*armA*) found in Poland was identified in *K. pneumoniae* in **France**

(Galimand M et al. 2003 AAC 47:2565)
Several new genes

<table>
<thead>
<tr>
<th>Gene</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ArmA</td>
<td></td>
</tr>
<tr>
<td>RmtB</td>
<td></td>
</tr>
<tr>
<td>RmtD</td>
<td>several Enterobacteriaceae – widely distributed</td>
</tr>
<tr>
<td>NpmA</td>
<td><em>E. coli</em> – Japan</td>
</tr>
<tr>
<td>RmtE</td>
<td><em>E. coli</em> – bovin</td>
</tr>
</tbody>
</table>

High frequency (75%) in NDM-1 producers

### Resistance genes – *A. baumannii* in Abu Dhabi, 2008

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>OXA23</th>
<th>Int</th>
<th>AmpC-IsAb1</th>
<th>PER</th>
<th>aadA</th>
<th>aac(3)-Ia</th>
<th>aac(3)-Ib</th>
<th>aac(6')-Ib</th>
<th>aac(6')-Ih</th>
<th>aph(3')-Ia</th>
<th>aph(3')-VI</th>
<th>strAB</th>
<th>ant(2')-Ia</th>
<th>armA</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td>110</td>
<td>76.4</td>
<td>62.7</td>
<td>30.9</td>
<td>14.5</td>
<td>34.5</td>
<td>12.7</td>
<td>6.4</td>
<td>14.5</td>
<td>22.7</td>
<td>35.5</td>
<td>35.5</td>
<td>46.4</td>
<td>22.7</td>
<td>27.3</td>
</tr>
<tr>
<td>Sporadic</td>
<td>30</td>
<td>16.7</td>
<td>30.0</td>
<td>23.3</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>6.7</td>
<td>20.0</td>
<td>23.3</td>
<td>10.0</td>
<td>30.0</td>
<td>3.3</td>
<td>6.7</td>
<td></td>
</tr>
<tr>
<td>Outbreak</td>
<td>80</td>
<td>98.8</td>
<td>75.0</td>
<td>33.8</td>
<td>20.0</td>
<td>38.8</td>
<td>17.5</td>
<td>8.8</td>
<td>17.5</td>
<td>23.8</td>
<td>40.0</td>
<td>43.8</td>
<td>52.5</td>
<td>30.0</td>
<td>35.0</td>
</tr>
</tbody>
</table>

*Note: The highlighted cell indicates the specific resistance gene's percentage in the outbreak scenario.*
### Resistance genes – *A. baumannii* in Abu Dhabi, 2008

<table>
<thead>
<tr>
<th>CLONE</th>
<th>N</th>
<th>„AMINOGYCOSIDE GENOTYPE“</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>2</td>
<td><em>aadA, (aac(3)-Ia), (aac(6’)-Ih), (aph(3’)-Ia), aph(3’)-VI, ant(2’)-Ia,</em></td>
</tr>
<tr>
<td>C</td>
<td>3</td>
<td><em>aadA, aac(6’)-Ih, aph(3’)-Ia, (aph(3’)-VI)</em></td>
</tr>
<tr>
<td>D</td>
<td>11</td>
<td><em>aadA, aac(6’)-Ih, aph(3’)-Ia, (aph(3’)-VI)</em></td>
</tr>
<tr>
<td>E</td>
<td>3</td>
<td><em>aph(3’)-VI, ant(2’)-Ia</em></td>
</tr>
<tr>
<td>F</td>
<td>19</td>
<td><em>aph(3’)-VI, ant(2’)-Ia</em></td>
</tr>
<tr>
<td>H</td>
<td>10</td>
<td><em>(aac(3)-Iia), (armA), strAB,</em></td>
</tr>
<tr>
<td>I</td>
<td>4</td>
<td><em>(armA), strAB,</em></td>
</tr>
<tr>
<td>J</td>
<td>12</td>
<td><em>(aadA), (aac(6’)-Ib), (aph(3’)-Ia), (armA), strAB,</em></td>
</tr>
<tr>
<td>L</td>
<td>2</td>
<td><em>aadA, aac(6’)-Ib, aac(6’)-Ih, aph(3’)-Ia, armA, strAB</em></td>
</tr>
<tr>
<td>M</td>
<td>4</td>
<td><em>(aac(3)-Ia), strAB,</em></td>
</tr>
<tr>
<td>N</td>
<td>10</td>
<td><em>(aac(3)-Ia), strAB,</em></td>
</tr>
</tbody>
</table>

(gene present in ≥50% of the strains)
Strains carrying *armA*

<table>
<thead>
<tr>
<th>MIC (mg/L)</th>
<th>AKC</th>
<th>GMC</th>
<th>STR</th>
<th>KAN</th>
<th>NET</th>
<th>SPEC</th>
<th>TOB</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;256</td>
<td>&gt;256</td>
<td>&gt;1024</td>
<td>&gt;1024</td>
<td>&gt;256</td>
<td>&gt;256</td>
<td>&gt;1024</td>
<td>&gt;1024</td>
</tr>
</tbody>
</table>
armA was likely to be introduced horizontally from a non-pathogenic Gram negative
The high GC% of rmtA suggests introduction from Actinomycetes (?)

In clinically relevant strains (Enterobacteriaceae, Non-fermenters)

- Plasmid-coded
- Transposon – associated
- Often in outbreak-causing strains

- Frequent association with aminoglycoside-modifying enzymes
  - ESBLs
  - Carbapenemases
  - Other R genes

- The resistance is high level (high MIC)
- The resistance spectrum is broad
  (practically all clinically useful aminoglycoside but streptomycin)
Laboratory diagnosis

When to suspect armA (or 16S methylases)?

If resistant to several aminoglycosides
If MICs are very high

**Problem** - not many are tested routinely
  - in automated systems MICs close to the breakpoints are tested

**Screening**

Disc diffusion with Gentamicin, Amikacin (and Arbekacin)
No zone of inhibition
Genta + Ami = 60% predictive value
+ Arbekacin = 90% predictive value

*Doi Y and Arakawa Y 2007 CID 46:88-94*

**Confirmation**

PCR

Laboratory diagnosis

**Aminoglycoside Resistance and Susceptibility Testing Errors in Acinetobacter baumannii-calcoaceticus Complex**

Kevin S. Akers,¹,² Chris Chaney,² Alice Barsoumian,² Miriam Beckius,³ Wendy Zera,¹,⁶ Xin Yu,³ Charles Guymon,⁴ Edward F. Keen III,⁵ Brian J. Robinson,⁵ Katrin Mende,¹‡ and Clinton K. Murray¹,²*

**Brief Communication:**
False Susceptibility to Amikacin by VITEK 2 in Acinetobacter baumannii Harboring armA

Sunkyung Jung, Jin Kyung Yu, Sang Hyun Shin, Kang Gyun Park, Dong Wook Jekarl, Kyungja Han, and Yeon-Joon Park
Laboratory Medicine Department, St. Mary’s Hospital, The Catholic University of Korea, Seoul, Korea
Conclusions

• The recent emergence and spread of 16S methylase-based aminoglycoside resistance is a clear and present danger among Enterobacteriaceae and non-fermenters

• The genes spread easily as mostly located on plasmids

• The cause a high level, broad spectrum resistance practically removing all aminoglycosides from the armory to treat these infections

• They are often associate with other resistance genes as ESBLs or carbapenemases

• The susceptibility pattern and high MIC could be suggestive, confirmation is by PCR

• Automated susceptibility testing, at least for amikacin in *Acinetobacter* could be biased
Thank you