Educational Workshop

EW12: Carbapenem resistance in non-fermenters

arranged with ESGARS

(ESCMID Study Group for Antimicrobial Resistance Surveillance)

Convenors:
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Gian Maria Rossolini (Siena, IT)

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Dora Szabo (Budapest, HU)
Alkis Vatopoulos (Athens, GR)
The role of beta-lactamases
_Gian Maria Rossolini (Siena, IT)_

No presentation has been submitted.
Martinez-Martinez – Role of efflux/influx mechanisms

Educational Workshop 12
Carbapenem resistance in non-fermenters

The role of efflux/influx mechanisms

Luis Martinez-Martinez
Service of Microbiology
University Hospital Marques de Valdecilla
Santander, Spain

Influx:
- Decreased penetration
- Altered Permeability
- Porin loss, structural changes of porins
- Altered LPS...

Efflux:
- Increased elimination
- Pumps: Energy consumption

USUALLY BOTH MECHANISMS ARE LINKED

*Pseudomonas aeruginosa*
*Acinetobacter baumanii*
*Stenotrophomonas maltophilia*
*Other Non-fermenters*

Pseudomonas aeruginosa
Martinez-Martinez – Role of efflux/influx mechanisms

Diffusion of Carbapenems through Specific Porin: OprD

*P. aeruginosa
*Several OprD-like proteins identified
*Specific: Basic aminoacids and gluconate

OprD EXPRESSION AND CARBAPENEM RESISTANCE IN P. aeruginosa

<table>
<thead>
<tr>
<th>MIC IMP</th>
<th>OprD</th>
<th>OprD</th>
<th>OprD</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;=1</td>
<td>5</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>4-8</td>
<td>6</td>
<td>8</td>
<td>5</td>
</tr>
<tr>
<td>&gt;=16</td>
<td>0</td>
<td>0</td>
<td>55</td>
</tr>
</tbody>
</table>

Dib et al. EJCMID 1995
### Martinez-Martinez – Role of efflux/influx mechanisms

#### AmpC/OprD IN *P. aeruginosa*

<table>
<thead>
<tr>
<th></th>
<th>AmpC</th>
<th>OprD</th>
<th>IMP</th>
<th>MPM</th>
<th>CRB</th>
<th>CAZ</th>
</tr>
</thead>
<tbody>
<tr>
<td>BASAL</td>
<td>+</td>
<td>1</td>
<td>0.25</td>
<td>64</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>DEREPRESSED</td>
<td>+</td>
<td>1</td>
<td>0.25</td>
<td>128</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td>BASAL</td>
<td>-</td>
<td>0.5</td>
<td>1</td>
<td>32</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>DEREPRESSED</td>
<td>-</td>
<td>16</td>
<td>2</td>
<td>64</td>
<td>32</td>
<td></td>
</tr>
</tbody>
</table>

#### ROLE OF OprD Loop7 IN THE ACTIVITY OF CARBAPENEMS AGAINST *P. aeruginosa*

<table>
<thead>
<tr>
<th>STRAIN</th>
<th>OprD</th>
<th>IMP Lys (+/-)</th>
<th>MPM Lys (+/-)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAO1</td>
<td>+</td>
<td>0.25/2</td>
<td>0.03/0.5</td>
</tr>
<tr>
<td>PSAE1</td>
<td>-</td>
<td>2/8</td>
<td>1/2</td>
</tr>
<tr>
<td>PSA1pDwt</td>
<td>+</td>
<td>0.25/1</td>
<td>0.13/2</td>
</tr>
<tr>
<td>PSA1pDmut</td>
<td>+</td>
<td>0.25/1</td>
<td>0.03/0.5</td>
</tr>
</tbody>
</table>

Epp et al, AAC 2001

#### MICs (mg/L) OF IMIPENEM AGAINST CLINICAL ISOLATES OF *P. aeruginosa* IN MUELLER-HINTON AND HUMAN SERUM

<table>
<thead>
<tr>
<th>STRAIN</th>
<th>Mueller-Hinton Broth</th>
<th>Human Serum</th>
<th>Human Serum +10 mM Lys</th>
</tr>
</thead>
<tbody>
<tr>
<td>433</td>
<td>0.78</td>
<td>0.05</td>
<td>0.1</td>
</tr>
<tr>
<td>638</td>
<td>1.56</td>
<td>0.1</td>
<td>0.39</td>
</tr>
<tr>
<td>1008</td>
<td>3.13</td>
<td>0.1</td>
<td>0.39</td>
</tr>
<tr>
<td>1609</td>
<td>1.56</td>
<td>0.1</td>
<td>0.2</td>
</tr>
<tr>
<td>1872</td>
<td>0.78</td>
<td>0.05</td>
<td>0.1</td>
</tr>
<tr>
<td>2093</td>
<td>1.56</td>
<td>0.2</td>
<td>0.39</td>
</tr>
<tr>
<td>2290</td>
<td>1.56</td>
<td>0.39</td>
<td>0.39</td>
</tr>
<tr>
<td>3359</td>
<td>3.13</td>
<td>0.2</td>
<td>0.39</td>
</tr>
<tr>
<td>3596</td>
<td>1.56</td>
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<td>0.39</td>
</tr>
<tr>
<td>6361</td>
<td>1.56</td>
<td>0.2</td>
<td>0.39</td>
</tr>
<tr>
<td>6394</td>
<td>1.56</td>
<td>0.39</td>
<td>0.39</td>
</tr>
<tr>
<td>8465</td>
<td>1.56</td>
<td>0.2</td>
<td>0.39</td>
</tr>
</tbody>
</table>

Fukuoka et al. AAC 1993
Martinez-Martinez – Role of efflux/influx mechanisms

**Loss of OprD porin in *P. aeruginosa***

- RNA expression of oprD
- PCR amplification of oprD

Walters et al, FEMS ML 2004

**ZINC DECREASES OprD LEVELS IN *P. aeruginosa*: RESISTANCE TO IMIPENEM**

- Conejo MC et al, AAC 2003

**EFFLUX SYSTEMS**

**MAJOR FAMILIES**

1. MFS: Major Facilitator Superfamily
2. RND: Resistance Nodulation-Division
3. SMR: Small Multidrug Resistance
4. ABC: ATP-Binding Cassette
5. MATE: Multidrug And Toxic Extrusion
EXPRESSION OF ACTIVE EFFLUX

1. BASAL EXPRESSION
2. OVEREXPRESSION

Active efflux is present in both (clinically) susceptible and resistant organisms

EFFLUX PROTEINS

Located in Cytoplasmic Membrane
Multisubstrate Systems (PUMP+MFP+OMF)
Multiple variants in the same host
Broad/Narrow substrate profile

OprM
P. aeruginosa
### MULTIDRUG EFFLUX PUMPS IN *P. aeruginosa*

<table>
<thead>
<tr>
<th>PUMP</th>
<th>MFP</th>
<th>OMP</th>
<th>GENES</th>
<th>REG</th>
<th>SUBSTRATES</th>
</tr>
</thead>
<tbody>
<tr>
<td>MexB</td>
<td>MesA</td>
<td>OprM</td>
<td>mexAmexBoprM</td>
<td>MexR</td>
<td>Tet, Cl, FQ, β-L (MPM,...),</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Ril, Nov, SXT,...</td>
</tr>
<tr>
<td>MexD</td>
<td>MexC</td>
<td>OprJ</td>
<td>mexCxexCoprJ</td>
<td>mexS</td>
<td>Tet, Cl, FQ, β-L (Cepheems), TMP</td>
</tr>
<tr>
<td>MexF</td>
<td>MexE</td>
<td>OprN</td>
<td>mexEmexForpN</td>
<td>mexT</td>
<td>Cl, TMP, FQ</td>
</tr>
<tr>
<td>MexY</td>
<td>MexX</td>
<td>OprM</td>
<td>mexXmexY</td>
<td>mexZ</td>
<td>AmGlu, Tet, Ery</td>
</tr>
</tbody>
</table>

### AmpC/OprM IN *P. aeruginosa*

<table>
<thead>
<tr>
<th>AmpC</th>
<th>OprM</th>
<th>IMP</th>
<th>MPM</th>
<th>CRB</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>+</td>
<td>1</td>
<td>0.5</td>
<td>25</td>
</tr>
<tr>
<td>+</td>
<td>-</td>
<td>1</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>-</td>
<td>+</td>
<td>0.25</td>
<td>0.25</td>
<td>25</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>0.25</td>
<td>&lt;0.015</td>
<td>0.125</td>
</tr>
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</table>

### OprM/OprD IN *P. aeruginosa*

<table>
<thead>
<tr>
<th>OprM</th>
<th>OprD</th>
<th>IMP</th>
<th>MPM</th>
<th>CRB</th>
</tr>
</thead>
<tbody>
<tr>
<td>+++</td>
<td>+</td>
<td>1</td>
<td>4</td>
<td>256</td>
</tr>
<tr>
<td>+++</td>
<td>-</td>
<td>16</td>
<td>16</td>
<td>256</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>1</td>
<td>0.5</td>
<td>64</td>
</tr>
<tr>
<td>+</td>
<td>-</td>
<td>16</td>
<td>4</td>
<td>64</td>
</tr>
<tr>
<td>-</td>
<td>+</td>
<td>1</td>
<td>0.125</td>
<td>1</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>16</td>
<td>0.5</td>
<td>2</td>
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</tbody>
</table>
### MICs of Imipenem against \(P.\ aeruginosa\) hyperproducing \(\text{MexCD-OprJ}\)

<table>
<thead>
<tr>
<th>Strain</th>
<th>OprD</th>
<th>MexC</th>
<th>IMP</th>
</tr>
</thead>
<tbody>
<tr>
<td>164</td>
<td>1.0</td>
<td>1.0</td>
<td>4</td>
</tr>
<tr>
<td>164-921C</td>
<td>1.3</td>
<td>736</td>
<td>0.5</td>
</tr>
<tr>
<td>164MI</td>
<td>1.0</td>
<td>1.0</td>
<td>4</td>
</tr>
<tr>
<td>164-MI-94C</td>
<td>0.8</td>
<td>332</td>
<td>1</td>
</tr>
<tr>
<td>164CD</td>
<td>1.0</td>
<td>1.0</td>
<td>8</td>
</tr>
<tr>
<td>164CD-921C</td>
<td>0.8</td>
<td>304</td>
<td>2</td>
</tr>
</tbody>
</table>

### ZINC AND RESISTANCE TO IMIPENEM.

**ROLE OF THE CzcCBA SYSTEM**

Sublethal [Zn] induce resistance to Zn and to IMP. Imipenem may also select for Zn-R.

Lethal [Zn] select stable mutants resistant to Zn and to IMP, because of mutation (V194L) in CzcS.

Expression of mutated CzcS causes increased expression of czcC and decreased expression of oprD.
Martinez-Martinez – Role of efflux/influx mechanisms

### MICs of Imipenem Against *P. aeruginosa* PAO1 and Derived Mutants in Different Media

<table>
<thead>
<tr>
<th></th>
<th>Mueller-Hinton</th>
<th>Minimal Medium</th>
<th>Minimal Medium+Lysine</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAO1</td>
<td>1.5</td>
<td>0.38</td>
<td>3</td>
</tr>
<tr>
<td>ΔOprD</td>
<td>8</td>
<td>3</td>
<td>8</td>
</tr>
<tr>
<td>MexAB+++</td>
<td>0.75</td>
<td>0.38</td>
<td>2</td>
</tr>
<tr>
<td>MexCD+++</td>
<td>0.75</td>
<td>0.38</td>
<td>3</td>
</tr>
<tr>
<td>MexXY+++</td>
<td>0.75</td>
<td>0.38</td>
<td>2</td>
</tr>
<tr>
<td>Const. AmpC</td>
<td>2</td>
<td>0.38</td>
<td>1</td>
</tr>
</tbody>
</table>

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### Acinetobacter baumannii

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### CarO Loss in *A. baumannii*

Resistant to carbapenems

Limanskaya AG; JCM 2002, 4776; Musai MA et al, AAC 2005, 1432
Martinez-Martinez – Role of efflux/influx mechanisms

33-36 kDa in *A. baumannii* and resistant to carbapenems

<table>
<thead>
<tr>
<th></th>
<th>IPM</th>
<th>MP</th>
<th>IPM</th>
<th>MP</th>
</tr>
</thead>
<tbody>
<tr>
<td>32</td>
<td>4</td>
<td>&gt;32</td>
<td>16</td>
<td>4</td>
</tr>
<tr>
<td>MPM</td>
<td>&gt;32</td>
<td>12</td>
<td>MPM</td>
<td>8</td>
</tr>
</tbody>
</table>

33-36 kDa in *A. baumannii* and resistant to carbapenems

OprD-like in *A. baumannii*

Porin HMP-AB of *A. baumannii*

Related to OmpA (E. coli) and OprF (P. aeruginosa)

<table>
<thead>
<tr>
<th>Super</th>
<th>HMP-AB of A. baumannii</th>
<th>OprF of E. coli</th>
<th>B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetate</td>
<td>1.50</td>
<td>0.80</td>
<td>A</td>
</tr>
<tr>
<td>Glucose</td>
<td>1.30</td>
<td>0.80</td>
<td>B</td>
</tr>
<tr>
<td>N-acetyl-d-glucosamine</td>
<td>2.25</td>
<td>0.70</td>
<td>C</td>
</tr>
<tr>
<td>Sucrose</td>
<td>1.30</td>
<td>0.70</td>
<td>D</td>
</tr>
<tr>
<td>Starch</td>
<td>0.04</td>
<td>0.80</td>
<td>E</td>
</tr>
</tbody>
</table>

*Gribun A et al, Curr Microbiol 2003, 434"
### Efflux systems in *A. baumannii*

<table>
<thead>
<tr>
<th>Efflux pump</th>
<th>Family</th>
<th>Substrate</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tet(A)</td>
<td>MFS</td>
<td>TET</td>
<td>Ribera et al, JAC 2003, 52:477</td>
</tr>
<tr>
<td>Tet(B)</td>
<td>MFS</td>
<td>TET, MIN</td>
<td>Martí S et al, EIMC 2006, 77</td>
</tr>
<tr>
<td>CmrA</td>
<td>MFS</td>
<td>CLOR</td>
<td>Fourtner PE et al, Proc Genet 2006, 62</td>
</tr>
<tr>
<td>AdeJK</td>
<td>RND</td>
<td>B-lac, CHL, TET, TIG, FG, ERY, CC, TMP, FG, RIF, MOY</td>
<td>Damier-Piolle et al, AAC 2008, 527</td>
</tr>
<tr>
<td>AbeM</td>
<td>MATE</td>
<td>AMG, FG, ERY, CHL, TMP</td>
<td>Su X et al, AAC 2000, 4382</td>
</tr>
</tbody>
</table>

### Efflux systems in *A. genomospecies 3*

<table>
<thead>
<tr>
<th>Efflux pump</th>
<th>Family</th>
<th>Substrate</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>AdeDE</td>
<td>RND</td>
<td>AMG, FQ, CHL, CAZ, CARBAP, RIF</td>
<td>Chau SL et al, AAC 2004, 4054</td>
</tr>
<tr>
<td>AdeXYZ</td>
<td>RND</td>
<td>??</td>
<td>Chu YW et al, JMM 2005, 477</td>
</tr>
</tbody>
</table>

### AdeABC in *A. baumannii*

<table>
<thead>
<tr>
<th>Strain</th>
<th>GEN</th>
<th>AMK</th>
<th>CTX</th>
<th>TET</th>
<th>CLO</th>
<th>PEF</th>
<th>OFX</th>
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</thead>
<tbody>
<tr>
<td>BM4454</td>
<td>2-8</td>
<td>8</td>
<td>16</td>
<td>64</td>
<td>64</td>
<td>255</td>
<td>128</td>
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<tr>
<td>BM4454-1</td>
<td>&lt;=0.25</td>
<td>1</td>
<td>4</td>
<td>8</td>
<td>128</td>
<td>16</td>
<td>4</td>
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</tbody>
</table>

Magnet S et al, AAC 2001, 3375
Active efflux involved in Carbapenem-resistance in *A. baumannii*?

<table>
<thead>
<tr>
<th>Strain</th>
<th>Initial MIC</th>
<th>MICs for Mutants</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>IMM</td>
<td>MPM</td>
</tr>
<tr>
<td>ab6</td>
<td>1</td>
<td>0.5</td>
</tr>
<tr>
<td>ab1254</td>
<td>3</td>
<td>0.75</td>
</tr>
</tbody>
</table>

*CCCP*; **Ccp**

---

A role for AdeABC in Carbapenem-resistance in *A. baumannii*?

---

Stenotrophomonas maltophilia
Martinez-Martinez – Role of efflux/influx mechanisms

**Efflux proteins in S. maltophilia K279a**

<table>
<thead>
<tr>
<th>Schematic ID</th>
<th>Name</th>
<th>Transporter or influx mechanism</th>
<th>Known regulator or efflux protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>smeN4998RF5</td>
<td>SmeABC</td>
<td>Type II efflux regulator (SmeABC)</td>
<td>3 efflux proteins were identified</td>
</tr>
<tr>
<td>smeN270RF5</td>
<td>SmeDF</td>
<td>Type I efflux regulator (SmeDF)</td>
<td>2 efflux proteins were identified</td>
</tr>
<tr>
<td>smeN5965</td>
<td>SmeJK</td>
<td>Type I efflux regulator (SmeJK)</td>
<td>1 efflux protein was identified</td>
</tr>
</tbody>
</table>

Crossman LC et al., Genome Biol 2008, 9:R74

**Susceptibility to meropenem and expression of smeABC and smeDEF**

<table>
<thead>
<tr>
<th>No.(%)</th>
<th>MICs against S. maltophilia K279a and mutants lacking RND efflux pump genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>smeABC (+)</td>
<td>55 (59%)</td>
</tr>
<tr>
<td>smeABC (-)</td>
<td>38</td>
</tr>
<tr>
<td>( P \text{ value} )</td>
<td>0.311</td>
</tr>
<tr>
<td>smeDEF (+)</td>
<td>29 (31%)</td>
</tr>
<tr>
<td>smeDEF (-)</td>
<td>64</td>
</tr>
<tr>
<td>( P \text{ value} )</td>
<td>0.009*</td>
</tr>
</tbody>
</table>

Chang L et al., JAC 2004, 53, 518

**MICs against S. maltophilia K279a and mutants lacking RND efflux pump genes**

<table>
<thead>
<tr>
<th>Strain</th>
<th>GEN</th>
<th>AMK</th>
<th>MPM</th>
<th>IMP</th>
<th>CLO</th>
<th>TET</th>
<th>CIP</th>
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</thead>
<tbody>
<tr>
<td>K279a</td>
<td>16</td>
<td>64</td>
<td>32</td>
<td>256</td>
<td>6</td>
<td>16</td>
<td>2</td>
</tr>
<tr>
<td>smeJ</td>
<td>8</td>
<td>32</td>
<td>32</td>
<td>256</td>
<td>6</td>
<td>8</td>
<td>1</td>
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<tr>
<td>smeK</td>
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<td>32</td>
<td>256</td>
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<td>smeJK</td>
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</tr>
<tr>
<td>smeZ</td>
<td>1</td>
<td>32</td>
<td>32</td>
<td>256</td>
<td>6</td>
<td>16</td>
<td>2</td>
</tr>
</tbody>
</table>

Crossman LC et al., Genome Biol 2008, 9:R74
Resistance to carbapenems is clearly related to porins (OprD) and efflux systems (MexAB-OprM) in *P. aeruginosa*.

In *A. baumannii*, several outer membrane proteins/porins are involved in carbapenem resistance, but the role of active efflux is still imprecise.

Little information is available on the actual importance of porins or active efflux in (carbapenem-resistant) *S. maltophilia*.
How to detect and characterize the resistant isolates

Carbapenem resistance in non-fermenters
Educational Workshop 12
19th European Congress of Clinical Microbiology and Infectious Diseases (ECCMID), Helsinki

Dora Szabo
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Carbapenem resistance

- Carbapenemase production
- Decreased permeability
- Efflux mechanisms

Carbapenemase production

- Chromosomal carbapenemases
  - Stenotrophomonas maltophilia
  - Aeromonas hydrophila
  - Aeromonas sobria
  - Cryobacterium spp.
  - Flavobacterium spp.
- Acquired carbapenemases
Acquired-carbapenemases

- **Class B metallo-beta-lactamases (MBLs)**
  - Most common in *Pseudomonas aeruginosa* and *Acinetobacter* spp.

- **Class A non metallo-beta-lactamases**
  - Rare in non-fermenters, in *Pseudomonas aeruginosa*

- **Class D non metallo-beta-lactamases**
  - Mainly in *Acinetobacter* spp.

**Class B Metallo-beta-lactamases - MBLs**

- Hydrolyze all beta-lactams, including carbapenems, with the exception of aztreonam
- High level resistance to carbapenems
- Inhibited by EDTA or thiol compounds
- Integron or transposon located

- **VIM-1**, *P. aeruginosa*, *Acinetobacter* spp.
- **IMP-1**, *P. aeruginosa*, *Acinetobacter* spp., *Achromobacter*
- **SPM-1**, *P. aeruginosa*
- **GIM-1**, *P. aeruginosa*
- **SIM-1**, *Acinetobacter* spp.

**Class D non metallo-beta-lactamases Oxacillinas**

- Hydrolyze all beta-lactams including carbapenems
- The level of hydrolytic activity is fairly weak compared with that of MBLs
- Chromosomal or plasmid encoded

- Three groups
  - **OXA-23** (includes OXA-27 and OXA-49)
  - **OXA-24** (includes OXA-25, OXA-26 and OXA-40)
  - **OXA-58**
Class A non metallo-beta-lactamases

- Can be broadly divided into five major groups:
  - GES-2 to GES-6; in *P. aeruginosa*;
    - Good carbapenemase activity
    - Inhibited by clavulanic acid
  - KPC-2; in *P. putida*;
    - Weak carbapenemase activity
    - Inhibited by clavulanic acid
  - (SME, IMI-, NMC-A; no recent reports, in Enterobacteriaceae)

Detection of carbapenemases

- Hodge test!!

Acquired MBLs in non-fermentative bacteria

- *P. aeruginosa* and *Acinetobacter* spp. are the most common hosts;
- *P. putida, P. stutzeri, Achromobacter* occur
- Worldwide distribution in *P. aeruginosa*
- Low prevalence, but regional outbreaks occur
Detection of Class B Metallo-beta-lactamases- MBLs

- Resistance phenotype: disc diffusion test or MIC values
  - Reduced susceptibility to carbapenems
  - Full resistance to all cephalosporins, sensitive to aztreonam

- Synergy tests based on MBL inhibition
  - MIC determination
  - Double disk synergy test
  - Combined disk test

Analysis of resistance phenotype

- Resistance phenotype: disc diffusion test or MIC values
  - Reduced susceptibility or resistance to carbapenems
  - Full resistance to cephalosporins,
  - Sensitive to aztreonam

- There is no CLSI or EUCAST recommendation for MBLs

Disk diffusion tests for MBL-producing *P. aeruginosa*

The isolate is resistant to imipenem, ceftazidime, piperacillin/tazobactam, ceftazidime, timentin, and susceptible to aztreonam
Szabo – How to detect and characterize resistant isolates

Inhibition zone diameters (in millimeters) around the aztreonam disk (30 µg) for the 84 metallo-ß-lactamase-carrying isolates.

Franklin et al. JCM 2006 44 (9):3139-3144

Synergy tests for MBLs based on MBL inhibition

- Synergy tests based on MBL inhibition
  - MIC determination
  - Double disk synergy test
  - Combined disk test
  - MBL inhibitors
    - EDTA !!
    - thiol-based compounds:
      - mercaptopropionic acid (MPA)
      - mercaptoacetic acid (SMA)
      - mercaptoethanol

MIC determination with inhibitors

- E-test
  - Imipenem strip +/- EDTA

- Microdilution test
19 (86.4%) of the 22 Pseudomonas isolates were positive by Etest.

- 60 carbapenem non-susceptible P. aeruginosa and Acinetobacter baumannii strains were tested;
  - all of them were positive by E-test
  - negative by PCR
  (Aktas SJID 2008;40(4):320-5)

**Microdilution test**

- Measuring imipenem MICs in the presence or absence of:
  - EDTA plus 1,10-phenanthroline (Migliavacca et al. 2002 JCM. 40:4388-4390)
  - Sodium mercaptoacetate (SMA) at 400 mg/mL (Hirakata et al. KJ 2008 82(4):285-91)
  - Dipicolinic acid at (DPA) 175 mg/ml (Hirakata et al. KJ 2008 82(4):285-91)

- The chelator mixture **reduce by fourfold or more the imipenem MICs** for MBL producers, while a lower effect or no effect with MBL nonproducers.
Double disk synergy test

Synergy between the EDTA disc placed next to imipenem, meropenem ceftazidime discs.

Double disk synergy tests with EDTA

- Imipenem + 750µg EDTA
  - Pseudomonas differentiated all MBL producing Pseudomonas
  - Acinetobacter spp. Sensitivity 95.7% and specificity 95%

- 60 carbapenem non-susceptible P. aeruginosa and A. baumannii strains were tested:
  - all of them were positive by E-test
  - negative by PCR
  - 0.5 M EDTA
    - 63.6% Pseudomonas positive
  - 100% Acinetobacter spp. positive
  - 0.1 M EDTA
    - 0% Pseudomonas positive
    - 7.7% Acinetobacter spp. positive

(Aktas SJID 2008;40(4):320-5)

Double disk synergy tests with MPA

- Ceftazidime + MPA
  - Ceftazidime for P. aeruginosa
  - Imipenem for Acinetobacter spp.
  - 2 cm distance MPA, 100% sensitivity and specificity
Szabo – How to detect and characterize resistant isolates

Double disk synergy tests with MPA

Hodge test by zinc sulfate (140 µg/disk) to an IPM disk.

In DDSTs, Pseudomonas EDTA disks were better, Acinetobacter MPA (3 µl) and SMA (3 mg) disks were better, EDTA (ca. 750 µg) plus SMA (ca. 2 mg) disks for both organisms.

CAZ-SMA DDSTs detected 72% of MBL-producing Acinetobacter spp.

Hodge test by zinc sulfate (140 µg/disk) to an IPM disk.

In DDSTs, Pseudomonas EDTA disks were better, Acinetobacter MPA (3 µl) and SMA (3 mg) disks were better, EDTA (ca. 750 µg) plus SMA (ca. 2 mg) disks for both organisms.

IPM disk + EDTA (750 µg) plus SMA (2 mg) disk for IPM-nonsusceptible isolates
Szabo – How to detect and characterize resistant isolates

**Combined disk (CD) test**

MBL producing *Pseudomonas aeruginosa*. The blank disc contains 750 μg EDTA. The zone of inhibition of imipenem, and meropenem is increased with the addition of EDTA indicating the presence of MBL.

Combined disk (CD) test

<table>
<thead>
<tr>
<th>Increase (in millimeters) in zone of inhibition around the imipenem-EDTA disk compared with the imipenem disk alone for 52 MBL-negative (MBL -ve) and 54 MBL-positive (MBL +ve) clinical isolates.</th>
</tr>
</thead>
<tbody>
<tr>
<td>MBL -ve</td>
</tr>
<tr>
<td>0</td>
</tr>
<tr>
<td>No. of isolates</td>
</tr>
<tr>
<td>0</td>
</tr>
</tbody>
</table>

Franklin et al. JCM 2006 44 (9):3139-3144

Combined disk (CD) test

Pseudomonas isolates

- ≥7-mm EDTA with FEP, FEP-CLA, CAZ, and CAZ-CLA
- Increasing the diameter the specificities were decreased to 60.0%, 70.0%, 43.3%, and 26.7%
Szabo – How to detect and characterize resistant isolates

Further characterization of the MBL-positive strains

- Isoelectric focusing
  - To determine the isoelectric points of beta-lactamases

- PCRs
  - Multiplex PCR

- Sequencing
Permeability changes

- Outer membrane protein, OprD, loss can cause imipenem resistance in *P. aeruginosa*
- OprD loss will effect only the imipenem susceptibility, not the meropenem
- Zink decreases the OprD level in *P. aeruginosa*
- The EDTA can interfere with the zink and influence the OprD expression causing interpretation problem in the screening tests.

Other resistance mechanism may mask or enhance the resistance !!!

- ESBL, AmpC-high and/or porin deficiency may affect aztreonam in MBL producing isolates or positive results in synergy tests
- ESBL + impermeability may produce carbapenem “resistance” with positive clavulanate synergy

Thank you for attention!
WHAT IS SURVEILLANCE?

The ongoing systematic collection, analysis, and interpretation of health data, essential to the planning, implementation and evaluation of public health practice, closely integrated with timely dissemination of these data to those who need to know.

SURVEILLANCE

- Must have a target
- Must be part of a procedure
- Must aim to a result
- Combine global and local data

- Health data
- Public health practice
- Health planning
- Health data
Alert System

- **A Surveillance System dedicated in the**
  **Early Recognition of an important new**
  **Public Health Thread.**

- **Can Be:**
  - Part of the existing (routine) surveillance System
  - An independent organization.

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**ESCMID STUDY GROUP REPORT**

European recommendations for antimicrobial resistance surveillance

- To detect new antimicrobial resistance mechanisms, and to develop continuously updated systems for interpretative reading of antibiotic susceptibility tests;
- To detect the threat of dissemination of especially unwanted resistance mechanisms or clones, e.g., methicillin-resistant S. aureus (MRSA), vancomycin-resistant enterococci (VRE), or extended-spectrum b-lactamases (ESBLs) in hospital wards, and multiresistant Mycobacterium tuberculosis or Strept. pneumoniae with high level penicillin resistance in the community;
- To serve as an inspiration for standardisation and harmonisation of antimicrobial susceptibility testing among laboratories taking part in the surveillance programme.

---

Alert System

- **To detect new antimicrobial resistance mechanisms,** and to develop continuously updated systems for interpretative reading of antibiotic susceptibility tests;
- **To detect the threat of dissemination of especially unwanted resistance mechanisms or clones,** e.g., methicillin-resistant S. aureus (MRSA), vancomycin-resistant enterococci (VRE), or extended-spectrum b-lactamases (ESBLs) in hospital wards, and multiresistant Mycobacterium tuberculosis or Strept. pneumoniae with high level penicillin resistance in the community.
Antibiotic Resistance.  
The surveillance targets

• Evolving trends in the incidence of antibiotic-resistant infections
• Evolving trends in the incidence of particular resistant clones
• Evolving trends in the incidence of particular mechanisms of resistance

Surveillance of Carbapenem resistance  
(Case Definition)

Do we survey:
• In vitro resistance (susceptibility data)?
• Presence of mechanisms?
• Both?
• Infection?
  – Invasive infection?
  – Infection and colonization?
• Resistance in animals

The type of resistance mechanism can result in differences:
• In clinical importance of this resistance
• In public Health significance
  – Mode of spread
  – Speed of spread
• The Built up of multiresistance
Mechanisms of carbapenem resistance

- Permeability
- Changes in penicillin-binding proteins
- Hyperproduction of AmpC + Porin loss

- Carbapenemases
  - Metallo-
  - Serine-
  - Other

Conclusion 1
Case definition of a resistant organism

- Two levels:
  - Clinical Resistance
    - Susceptibility testing
  - Mechanism
    - Type of carbapenemase

- Low sensitivity and specificity. Important in Clinical Practice
- Increase sensitivity and specificity
- Higher relevance in Public Health

Surveillance networks

- National
- Regional
  - “Private”
    - Pharmaceutical companies

International collaboration
Europe

- EARSS (ECDC)
  - MIC data

6. EARSS protocol for testing of Pseudomonas aeruginosa

- Objective: To determine the proportion of P. aeruginosa resistant to piperacillin, carbenapenem, thienamycines and ceftazidime (in blood and CSF cultures).

5.1. EARSS requirement

Report microbicidal susceptibility to AST results for the primary actions of P. aeruginosa from non-biological (i.e., for patients, see paragraph 5) in the study.

5.2. Test procedures

[Diagram showing test procedures]
Surveillance of Antibiotic Resistance in Greece

   1. To report resistance rates
   2. The Early Warning System.
      1. To trace (new) mechanisms of resistance.
      2. To trace the spread of resistance

What resistance rates represent?

- Resistance rates per organism
  - Per Hospital
  - Per Department
  - Per region
  - Per country
- Incidence rates (Infections):
  - Per hospital
  - Per department
  - Per region
  - Per country
- Number of Hospitals with (resistant) infections
Presentation of results

<table>
<thead>
<tr>
<th>Prevalence (resistance rates)</th>
<th>Incidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>• <em>Does not</em> represent burden of disease.</td>
<td>– Represents burden of disease</td>
</tr>
</tbody>
</table>

Relative incidence

relative rate of isolation of the respective resistant bacterial species in the respective hospital.

How do we epidemiologically characterize a country?
Surveillance of resistance

- **VIM (+)** *P. aeruginosa* is isolated in Greece
- **VIM (+)** *P. aeruginosa* is isolated from (at least) 1 hospital

- **VIM (+)** *P. aeruginosa* is being established in Greece
- **VIM (+)** *P. aeruginosa* is isolated from many hospitals

Surveillance of resistance

- **Epidemic**
  - Monoclonal epidemic
  - Polyclonal epidemic *allodemic* (allos = other)

- **Endemic**
  - Polyclonal (?)
Understanding the spread

- Typing and elucidating of clones
- PFGE
- Other methods
  - MLST
- Commercially available systems
- Studying the genetic environment
  - Plasmids
  - Transposon elements
    - Integrons
- Other resistance traits
  - Virulence traits?

Deciding upon a system

- Possibility to create a data base
  - MLST
  - PFGE
  - DiversiLab™
- Macro VS Local epidemiology
  - MLST
  - PFGE
  - PCR systems
- Cost
KPC-2 \(\beta\)-Lactamase producing *Klebsiella pneumoniae* infections in Greek hospitals are mainly due to an hyperepidemic clone.
Studying the genetic environment

- To predict possible spread among strains
- To predict the built up of multiresistance
- To predict the public health importance of a mechanism

Integrons harboring bla<sub>VIM</sub> genes

<table>
<thead>
<tr>
<th>Species</th>
<th>Gene 1</th>
<th>Gene 2</th>
<th>Gene 3</th>
<th>Gene 4</th>
<th>Gene 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>P. aeruginosa</td>
<td>aac(6')-IIc</td>
<td>intI</td>
<td>sulI</td>
<td>qacEΔ1</td>
<td>rhlB</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>aacA4</td>
<td>intI</td>
<td>sulI</td>
<td>qacEΔ1</td>
<td>rhlB</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>aacA7</td>
<td>intI</td>
<td>sulI</td>
<td>qacEΔ1</td>
<td>rhlB</td>
</tr>
<tr>
<td>E. coli</td>
<td>mecA</td>
<td>blaoxa-2</td>
<td>qacEΔ1</td>
<td>sulI</td>
<td>rhlB</td>
</tr>
<tr>
<td>E. coli</td>
<td>mecA</td>
<td>blaoxa-2</td>
<td>qacEΔ1</td>
<td>sulI</td>
<td>rhlB</td>
</tr>
</tbody>
</table>

Conclusion 2
Surveillance Systems

**Three levels:**

1. Based on susceptibility data
2. Detecting mechanisms and surroundings
3. Detecting mechanisms in clones.
   - High risk clones?
Conclusion 2
Surveillance Systems

Assessing the burden of disease

• Infections or colonizations?
  – Virulence of strains?

• Implication in outbreaks?

A surveillance system must also assess the magnitude of the problem:

• Denominator data
  • Incidence
  • Prevalence
  – Resistance rates

The importance of quick and efficient international communication

Tracking the international spread of clones