Strategies to design new antibacterial drugs

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Hospital Clinic, Barcelona
**Burden of multidrug-resistant bacteria in the European Union**

**Human burden**

- Infection (6 most frequent MDR bacteria, 4 main types of infection) approx. 400,000 / year
- Attributable deaths approx. 25,000 / year
- Extra hospital days approx. 2.5 million / year

**Economic burden**

- Extra in-hospital costs approx. € 1 billion / year
- Productivity losses approx. € 600 million / year

Source: ECDC, 2009
TACKLING DRUG-RESISTANT INFECTIONS GLOBALLY: AN OVERVIEW OF OUR WORLD

THE REVIEW ON ANTIMICROBIAL RESISTANCE
CHAIR BY JIM O’NEILL

JANUARY 2016
How to diminish the emergence and spread of antimicrobial resistance bacteria?

- **Antimicrobial stewardship**
  - Leadership commitment and appointing AS team
  - Rapid and affordable diagnostic (Diagnostic stewardship)
  - Systematic evaluation of on-going treatment
  - Monitoring antibiotic prescribing and resistance patterns
  - Reporting information on antibiotic use and resistance
  - Education of clinicians about resistance and optimal prescribing

- **Control**
  - Active screening of contacts of MDR-GNB patients should be decided on the background of prevalence in individual hospitals.
  - Screening of patients upon admission
  - Barrier isolation and isolation in single rooms or cohort isolation are implemented on the basis of inpatient risk areas.
  - Hand hygiene
  - Environmental cleaning
  - Decolonization or sensitization of the MDRB

- **General Population**
  - Adhere strictly to therapeutic schemes
  - Respect proper treatment duration
  - Avoid self-medication with antibiotics
  - Proper cooking and handling of food
  - Hand-washing, when:
    - Before eating
    - Before and after touching a sick person
    - After using the bathroom
    - After touching an animal or handle animal waste
    - After handling rubbish
    - After being in places frequented by many people (example: public transportation)

- **Pharmacists**
  - Reject to sale without prescription
  - Inform patients about when are antibiotics needed, how to take them correctly and the consequences of a misuse.

- **Medical doctors**
  - Practice safe prescription of antibiotics
  - Use of point-of-care tools to ensure when are antibiotics needed

- **Veterinarians**
  - Reduce the use of antibiotics in livestock
  - Avoid using antibiotics as prophylaxis
  - Provide veterinarians with latest information regarding antimicrobial resistance

- **Antimicrobial resistance surveillance**
  - At a national level
  - At an international level

- **Antibiotic consumption**
  - At a national level
  - At an international level

- **Defining integrated plans**
  - At a national level
  - At a global level

- **Implementation of action plan**
  - In each country
  - In terms of education strategies among General Population as well as among Health specialists and Veterinarians

Vila J (2018) CMI 24: 684
Boucher HW, et al.  
Bad Bugs, No Drugs: No ESKAPE! An Update from the Infectious Diseases Society of America  
Clinical Infectious Diseases 2009; 48:1-12

Enterococcus faecium  
Staphylococcus aureus  
Klebsiella pneumoniae  
Acinetobacter baumannii  
Pseudomonas aeruginosa  
Enterobacter spp

No newly approved classes of antibiotics have been discovered since 1962 for the most dangerous types of bacteria – Gram negatives.

No new classes at all discovered after 1984.

Ten new ANTIBIOTICS by 2020
Priority 1: CRITICAL

*Acinetobacter baumannii*, carbapenem-resistant

*Pseudomonas aeruginosa*, carbapenem-resistant

*Enterobacteriaceae*, carbapenem-resistant, 3rd generation cephalosporin-resistant
1. Derivative of known antibacterial agents
Derivative of known antimicrobial agents

- Modification of the basic structure of the antimicrobial agent which circumvents antibacterial resistant mechanisms
Derivative of known antimicrobial agents

- Modification of the basic structure of the antimicrobial agent which circumvents antibacterial resistant mechanisms
- Development of a compound inhibiting the mechanisms of resistance for an antibacterial agent
Derivative of known antimicrobial agents

Blocking antibacterial resistance mechanisms

- Inhibition of beta-lactamases
- Inhibition of efflux pumps
- Inhibition of aminoglycoside-modifying enzymes
- Blocking the SOS response
Derivative of known antimicrobial agents

Blocking antibacterial resistance mechanisms

- Inhibition of beta-lactamases

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<table>
<thead>
<tr>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Clavulanic acid, tazobactam, sulbactam</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>carbapenemases</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>AmpC</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>carbapenemasas</td>
<td></td>
</tr>
</tbody>
</table>

Avibactam: inhibits class A and C and some class D enzymes
Derivative of known antimicrobial agents

Blocking antibacterial resistance mechanisms

- Inhibition of beta-lactamases
- Inhibition of efflux pumps
- Inhibition of aminoglycoside-modifying enzymes
- Blocking the SOS response
Vila J, Fàbrega A, Roca I, Hernández A, Martínez JL
Efflux pumps as an important mechanism for quinolone resistance
Adv Enzymol Relat Areas Mol Biol 2011: 77; 167-235
Efflux pump of the RND family found in Enterobacteriaceae

Efflux pump inhibitor RND (Preclinical phase)

MP-601,205
Design of antimicrobial drugs

Blocking efflux pumps
Exoris collection
Cyclic peptides
1. Derivative of known antibacterial agents
2. New antibacterial agents

bacteria
DISCOVERY OF NEW ANTIBACTERIAL AGENT

• Classical
  • Secondary metabolites of bacteria and fungi with antimicrobial activity
  • Plant extracts
  • Marine macro and microorganisms
DISCOVERY OF NEW ANTIBACTERIAL AGENT

• Genomic
  – New tools on chemistry and molecular biology
    • Genomics and recombinant DNA.
    • Molecular modeling
    • Combinatorial chemistry and chemical structure.
Criteria of selection

Genome sequences

Genome model: *E.coli*

Genes

246 very conserved genes in all species

68 no found in humans

18 Essentials

16 Non essentials

34 Unknown

3 New protein targets

H.influenzae

Mycoplasma

S.pneumoniae

C.pneumoniae

P.aeruginosa

Comparison with human sequence

Lost of the function

Viability

Function of the gen

Essentiality

# Inhibitors of new protein targets (Phase 1/2*)

June 2018, 42 antibioitcs in clinical development

<table>
<thead>
<tr>
<th>Antibacterial agent</th>
<th>“Target”</th>
<th>Pharma. Indust.</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRS3123</td>
<td>Methionyl-tRNA synthetasa</td>
<td>Crestone Inc.</td>
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<tr>
<td>MGB-BP-3</td>
<td>DNA minor groove</td>
<td>MGB Biopharma</td>
</tr>
<tr>
<td>CG400549*</td>
<td>Biosynthesis of fatty acids (FabI)</td>
<td>CrystalGenomics</td>
</tr>
<tr>
<td>Afabicin*</td>
<td>Biosynthesis of fatty acids (FabI)</td>
<td>Debiopharm Int.</td>
</tr>
<tr>
<td>Ramoplanin*</td>
<td>Lipid I, II</td>
<td>Nanotherapeutics</td>
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</tbody>
</table>
# Inhibitors of new protein targets (Phase 2/3*)

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<th>Antibacterial agent</th>
<th>“Target”</th>
<th>Pharma. Indust.</th>
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<tr>
<td>Cadazolid</td>
<td>Oxazol.-quinolone hybrid</td>
<td>Actelion Pharm.</td>
</tr>
<tr>
<td>Cefiderocol*</td>
<td>Siderophor-cephalosporin</td>
<td>Shionogi Co.</td>
</tr>
<tr>
<td>Murepavidin*</td>
<td>LptD**</td>
<td>Polyphor AG.</td>
</tr>
</tbody>
</table>

Specific of *P. aeruginosa**
1. Derivative of known antibacterial agents
2. New antibacterial agents
3. Blockers of virulence factors
Antivirulence drugs: A therapeutic alternative?

- In the presence of these compounds, bacterial pathogens will have difficulties to generate an infection and will therefore be more easily eliminated by immune systems.

- Proposed targets for antivirulence drugs:
  - Fimbria (GNB), Surface proteins (GPB)
  - Toxins
  - Type III secretion systems
  - Quorum sensing
  - Regulation of virulence factors:
    - Two component systems; e.g., *Enterococcus*, the vanA gene is regulated by vanR-vanS

- Monoclonal antibodies
OmpA a virulence factor of *A. baumannii*

ompA is a highly conserved protein specially in gram-negatives bacteria.

10-13 Å is the size of the pore

3 domains (intracellular, intermembrane, transmembrane)

Union to Fibronectin
- Protein homologue using I-TASSER (with and without *E. coli* template)

- Perform docking experiments of the protein homologue using all the compounds from the EXORIS library (with Dr. Martin Ivanov)
Interaction OmpA – cyclic peptide
**OmpA inhibitors**
- No cytotoxic
- No antimicrobial activity

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Cell viability (%)</th>
<th>MIC (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.25 mg/mL</td>
<td>0.5 mg/mL</td>
</tr>
<tr>
<td>MV6</td>
<td>98.89±0.41</td>
<td>99.69±0.26</td>
</tr>
<tr>
<td>MV5</td>
<td>98.09±0.27</td>
<td>98.33±0.40</td>
</tr>
<tr>
<td>MV3B</td>
<td>99.47±0.39</td>
<td>99.77±0.17</td>
</tr>
<tr>
<td>MV8</td>
<td>99.27±0.21</td>
<td>99.7±0.14</td>
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<tr>
<td>MV9</td>
<td>98.86±0.08</td>
<td>100.05±0.39</td>
</tr>
<tr>
<td>MV10</td>
<td>98.31±0.35</td>
<td>99.14±0.44</td>
</tr>
<tr>
<td>NBA010011</td>
<td>98.17±0.60</td>
<td>98.79±0.28</td>
</tr>
</tbody>
</table>
Calculation of bacterial adherence

**MV6** (&Arg-D-Pro-Trp-Arg-D-Pro-Trp&)

**MV5** (&Trp-D-Pro-Arg-Trp-D-Pro-Arg&)

**MV3B** (&D-Arg-Pro-Trp-D-Arg-Pro-Trp&)

**MV8** (&Arg-Pro-D-Trp-Arg-Pro-D-Trp&)

<table>
<thead>
<tr>
<th>ATCC 17978</th>
<th>0.25 mg/ml</th>
<th>0.5 mg/ml</th>
<th>1 mg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MV6</strong></td>
<td></td>
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Negative Control

Calculation of bacterial adherence

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<tbody>
<tr>
<td>SXV4</td>
<td>98.9 ± 0.45</td>
<td>99.5 ± 0.26</td>
<td>100 ± 0.37</td>
<td>&gt;500</td>
</tr>
</tbody>
</table>

Peptide concentrations | Cell viability (%) | MIC (mg/L) | SXV4 (Ac-Trp-D-Pro-Arg-Trp-D-Pro-Arg-OH)
Murine sepsis peritoneal model

A. baumannii

Spleen

Lung

1. Derivative of known antibacterial agents
2. New antibacterial agents
3. Blockers of virulence factors
4. Nanoparticles and Antibacterial peptides / peptidomimetics
NANOBIOCIDES

- Metal and metal oxides e.g. nAg, ZnO, CuO, TiO$_2$
- Engineered/synthesized nanoparticles such as fullerenes, e.g. nanomagnetite (nC$_{60}$) and carbon nanotubes
<table>
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<tr>
<th>Antibacterial agent</th>
<th>Description</th>
<th>Indication</th>
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<tr>
<td>Omiganan</td>
<td>Indolicidin</td>
<td>Topical, anti-acne</td>
</tr>
<tr>
<td>Isegana</td>
<td>Protegrin</td>
<td>Failed</td>
</tr>
<tr>
<td>Locilex</td>
<td>Pexiganan</td>
<td>Topical, diabetic food</td>
</tr>
<tr>
<td>PAC-113</td>
<td>Histatin</td>
<td>Topical, mucositis</td>
</tr>
<tr>
<td>LTX-109</td>
<td>Synthetic mimetic</td>
<td>Topical, MRSA</td>
</tr>
<tr>
<td>DPK-060</td>
<td>LL37-variant</td>
<td>Topical, atopic dermatitis</td>
</tr>
<tr>
<td>PMX-30067</td>
<td>Defensin core mimetic</td>
<td>Intravenous, MRSA</td>
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CERAGENINS

• Cholic Acid-Based Mimics of Antimicrobial Peptides
## CERAGENINS

<table>
<thead>
<tr>
<th>Ceragenin (mg/L)</th>
<th>A. baumannii</th>
<th>K. pneumoniae</th>
<th>P. aeruginosa</th>
</tr>
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<tbody>
<tr>
<td>CSA 138</td>
<td>2</td>
<td>16</td>
<td>1</td>
</tr>
<tr>
<td>CSA 13</td>
<td>4</td>
<td>8</td>
<td>&lt;0.5</td>
</tr>
<tr>
<td>CSA 131</td>
<td>2</td>
<td>8</td>
<td>&lt;0.5</td>
</tr>
<tr>
<td>CSA 44</td>
<td>8</td>
<td>4</td>
<td>1</td>
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Vila-Farrés et al. (2015) IJAA 46: 568
### CERAGENINS

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<tbody>
<tr>
<td></td>
<td>MIC$_{50}$</td>
<td>MIC$_{90}$</td>
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<tr>
<td>CSA 138</td>
<td>2</td>
<td>4</td>
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<tr>
<td>CSA 13</td>
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15 colistin-susceptible (A. baumannii and P. aeruginosa) strains
TIME-KILLING CURVES

Killing curve A. baumannii colistin-R

log_{10} CFU/ml vs Time (h)

Killing curve P. aeruginosa colistin-R

log_{10} CFU/ml vs Time (h)

Vila-Farrés et al. (2015) IJAA 46: 568
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5. Bacteriophages and enzybiotics
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6. Antisense RNA