The significance of in vitro antibiotic resistance

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What is ‘antibiotic resistance’?

• ‘Antibiotic resistance’ means different things to different people:
  – Epidemiological resistance
  – Clinical microbiology resistance or *in vitro* resistance
  – Clinical resistance or *in vivo* resistance
What is ‘antibiotic resistance’?

- Epidemiological resistance:
  - Reduced susceptibility of bacteria to antibiotics due to the presence of resistance genes / mutations
  - Increase in MIC value / shift in MIC$_{50}$-MIC$_{90}$ values
What is ‘antibiotic’ resistance?

MIC distribution of *S. pneumoniae* in Belgium

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What is antibiotic resistance

• ‘antibiotic resistance’ means different things to different people:
  – Clinical microbiology resistance or ‘in vitro’ resistance
  • Breakpoint resistance
    – Reduced susceptibility of bacteria to antibiotics above predefined nationally / internationally accepted limits
  – Epidemiological resistance:
  – Clinical resistance or ‘in vivo’ resistance
What is ‘antibiotic’ resistance?

MIC distribution of *S. pneumoniae* in Belgium

Breakpoints: in vitro resistance

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Evolution of *S. pneumoniae* ‘in vitro’ resistance rates to penicillin (Belgium)

- peni-I: MIC > 0.1 and ≤ 1 (low level R)
- peni-R: MIC > 1 (high level R)
Determination of breakpoints

- Based on ill-defined mix of microbiological, pharmaco-kinetic, pharmaco-dynamic, clinical data
  - Periodically re-evaluated?
- Different organisations establish (different) breakpoints
  - USA: FDA, NCCLS
  - UK: BSAC; France: SFM, Germany: DIN, Spain, Sweden, The Netherlands
  - EUCAST
- Impact on treatment guidelines
## Breakpoints for enterobacteriaceae

<table>
<thead>
<tr>
<th>Country</th>
<th>MIC breakpoint, ug/mL</th>
<th>cefotaxime</th>
<th>ceftazidime</th>
</tr>
</thead>
<tbody>
<tr>
<td>United States</td>
<td>≤ 8</td>
<td>≤ 8</td>
<td></td>
</tr>
<tr>
<td>France</td>
<td>≤ 4</td>
<td>≤ 4</td>
<td></td>
</tr>
<tr>
<td>Norway</td>
<td>≤ 2</td>
<td>≤ 2</td>
<td></td>
</tr>
<tr>
<td>England</td>
<td>≤ 1</td>
<td>≤ 1</td>
<td></td>
</tr>
<tr>
<td>Sweden</td>
<td>≤ 0.5</td>
<td>≤ 2</td>
<td></td>
</tr>
<tr>
<td>Spain</td>
<td>≤ 1</td>
<td>≤ 1</td>
<td></td>
</tr>
</tbody>
</table>
Breakpoints for enterobacteriaceae:

ceftria MIC frequency distribution
inducible enterobacteriaceae NPRS 3,4,5,6

- 0.25: 68% S
- 0.5: 61% S
- 1: 56% S
- 2: 53% S
- 4: 61% S
- 8: 68% S
- 16: 68% S
- 32: 68% S
- 64: 53% S
- 128: 53% S
Breakpoint resistance and epidemiology of resistance

• ‘In vitro’ resistance or breakpoint resistance is often a crude and rather insensitive measure of reduced susceptibility of bacteria to antibiotics
• Different breakpoints hinder comparison between national resistance rates
What is ‘antibiotic resistance’?

– Epidemiological resistance
– Clinical microbiology resistance or in vitro resistance:
– Clinical resistance or in vivo resistance:
  • Increased risk of treatment failures
  • Breakpoints take into account local differences in dosage
‘In vivo’ significance of ‘in vitro’ penicillin-nonsusceptible S. pneumoniae pneumonia carries a higher mortality risk

- Antibiotic resistance (breakpoint resistance) is clinically not relevant

- Pneumococcal pneumonia
  - Antibiotic resistance (breakpoint resistance) is clinically not relevant
  - Penicillin-nonsusceptible S. pneumoniae pneumonia carries a higher mortality risk
‘In vivo’ significance of ‘in vitro’ penicillin resistance for pneumococcal pneumonia

- Probably no treatment failures
- Treatment failures

NCCLS breakpoints:
- S (sensitive)
- I (intermediate)
- R (resistant)

Penicillin MIC (mg/L):

- 0.03
- 0.06
- 0.12
- 0.25
- 0.5
- 1
- 2
- 4
- ≥8

No treatment failures

<table>
<thead>
<tr>
<th>Penicillin MIC (mg/L)</th>
<th>0.03</th>
<th>0.06</th>
<th>0.12</th>
<th>0.25</th>
<th>0.5</th>
<th>1</th>
<th>2</th>
<th>4</th>
<th>≥8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment failures</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>
‘In vivo’ significance of ‘in vitro’ ery- resistance in pneumococcal infections

• In vivo-in vitro paradox:
  – ‘In vivo’ success of macrolides/azalides despite in vitro resistance
    • Lynch ’02, Amsden ’99, Bisahi ’02
  – Treatment failure, breakthrough bacteremia
    • Leclerq ’02, Hyde ’01, Kelley ’00, Musher ’02, Kays ’02, Lonks ’02, Van Kerkhoven ’03, Butler ’03,
  – Failure to eradicate ery-R strains in acute otitis media
‘In vivo’ significance of ‘in vitro’ ery-resistance in pneumococcal infections

- In vitro susceptibility testing may overestimate resistance levels
  - Does not take into account
    - Tissue penetration
    - Additional non-antimicrobial effects
    - Host response
    - Low-level resistance (efflux) may be overcome by antibiotics
‘In vivo’ & ‘in vitro’ FQ-resistance in pneumococci

Table 1. Microbiologic characteristics of *Streptococcus pneumoniae* isolated before, during, or after therapy with oral levofloxacin from four patients with community-acquired pneumonia.

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Source and Time of Culture</th>
<th>Serotype</th>
<th>PFGE Pattern†</th>
<th>Susceptibility to Levofloxacin‡</th>
<th>Minimal Inhibitory Concentration§ (µg/ml)</th>
<th>Amino Acid Substitution</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Sputum, before treatment</td>
<td>23F</td>
<td>A</td>
<td>S</td>
<td>1 (S) 0.12 (S) 0.25 (S)</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Sputum, after treatment</td>
<td>23F</td>
<td>A</td>
<td>R</td>
<td>8 (R) 1 (S) 2 (I)</td>
<td>S79F S81F</td>
</tr>
<tr>
<td>2</td>
<td>Sputum, before treatment</td>
<td>6A</td>
<td>B</td>
<td>S</td>
<td>4 (I) 0.25 (S) 0.5 (S)</td>
<td>S79F</td>
</tr>
<tr>
<td></td>
<td>Sputum, during treatment</td>
<td>6A</td>
<td>B</td>
<td>R</td>
<td>16 (R) 4 (R) 4 (R)</td>
<td>S79F S81F</td>
</tr>
<tr>
<td>3</td>
<td>Blood, before treatment</td>
<td>14</td>
<td>C</td>
<td>R</td>
<td>16 (R) 4 (R) 2 (I)</td>
<td>S79F S81Y</td>
</tr>
<tr>
<td></td>
<td>Pleural fluid, during treatment</td>
<td>14</td>
<td>C</td>
<td>R</td>
<td>16 (R) 4 (R) 2 (I)</td>
<td>S79F S81Y</td>
</tr>
<tr>
<td>4</td>
<td>Sputum, during treatment</td>
<td>ND</td>
<td>ND</td>
<td>R</td>
<td>16 (R) 4 (R) 8 (R)</td>
<td>S79F D83Y S79Y E85K</td>
</tr>
</tbody>
</table>

*PFGE denotes pulsed-field gel electrophoresis, S susceptible, R resistant, I having intermediate susceptibility, and ND not done. Dashes indicate that no mutation was found.

†Unique PFGE patterns are designated by arbitrary single letters.
‡Susceptibility was tested by the disk-diffusion method.
§The degree of susceptibility is indicated in parentheses.

Davidson, N Eng J Med, ’02
‘In vivo’ significance of ‘in vitro’ FQ-resistance for pneumococcal pneumonia

• Increased risk of selecting FQ-resistant *S. pneu* during treatment if
  – *S. pneu* with reduced susceptibility (first step mutants)
  – Use of insufficiently active FQ’s (peak/MIC)

• Increased risk of clinical failure with FQ-resistant *S. pneu*

  » Perez-Trallero, Eur J Clin Microbiol Infect Dis, ’90;
    Davidson, NEJM, ’02, de la Campa, AAC, ’03, Perez-
    Trallero, EID, ‘03
Why is breakpoint resistance often a bad predictor of clinical outcome

• Microbiological (breakpoint) definition of resistance is imprecise
• Underlying host factors impact on infection outcome
• Dosage, target organ not taken into account
Why is breakpoint resistance often a bad predictor of clinical outcome

• In vitro susceptibility determination differs from in vivo situation
  – Small ‘in vitro’ inoculum size
    • $10^4$ CFU - $5.10^5$ CFU/ml or mm$^2$ vs $10^5$-$10^9$/ml or mm$^2$ in vivo
  – Constant antibiotic concentration for 16-18 hrs vs changing concentrations in vivo
conclusions

• Mix of criteria (clinical, pharmacological, epidemiological) or single criterion for establishing breakpoints?
• Patient / organ / organism dependent breakpoints?
• Regional breakpoints or internationally comparable breakpoints?
• Regular reassessment of breakpoints?
conclusions

• Other ‘in vitro’ methods to determine susceptibility that resemble more the ‘in vivo’ situation?
• Switch to MIC determination?