Expert rules in susceptibility testing

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Expert rules

- Intrinsic resistance
- Exceptional phenotypes (mainly resistance)
- Interpretive reading
Basis for rules

• Rules
  - Intrinsic resistance
  - Exceptional phenotypes (mainly resistance)

are based on analysis of in vitro data (MICs/breakpoints, frequencies of resistance..)
Interpretive reading is more complex

What is interpretive reading?

Inference of resistance mechanisms from susceptibility test results and interpretation of clinical susceptibility on the basis of the resistance mechanism
# Interpretive reading: the process

<table>
<thead>
<tr>
<th>Process</th>
<th>Example</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Test susceptibility</td>
<td>• <em>S. aureus</em> resistant to cefoxitin (oxacillin)</td>
</tr>
<tr>
<td>• Infer resistance mechanism</td>
<td>• Acquisition of the <em>mecA</em> gene</td>
</tr>
<tr>
<td>• Interpret clinical susceptibility on the basis of the resistance mechanism</td>
<td>• Report resistant to all β-lactams</td>
</tr>
</tbody>
</table>
Expert rules should be evidence based

• In particular for the interpretive rules since a « S » report may be changed to « I » or « R »
  → Decrease the number of available antibiotics

• Rules should be based on current evidence (microbiology, experimental models, clinical data)

• Evidence should be published

• Quality of evidence should be assessed

• Exceptions are possible and should be noted
Grading of evidence base for EUCAST Expert rules

**A**
Clinical evidence that reporting the test result as susceptible leads to clinical failures.

Evidence is weak and based only on a few case reports or on experimental models

**B**
No clinical evidence, but microbiological data suggest that clinical use of the agent should be discouraged

**C**
No clinical evidence, but microbiological data suggest that clinical use of the agent should be discouraged

No clinical evidence, but microbiological data suggest that clinical use of the agent should be discouraged
The objective of this presentation is to review evidence for some examples of rules.
E. coli producing CTX-M-15

Synergism between 3GC/aztreonam and clavulanic acid
EUCAST interpretive rule 9.1 (Enterobacteriaceae)

If R or I to any 3rd or 4th gen. oxyimino-cephalosporin or aztreonam, and positive for ESBL edit the S result for any of the oxy-iminocephi. and aztreonam as I and the I result as R

1. Evidence for this rule is A (in vitro data + experimental models + clinical data)
2. Evidence for this rule is B (in vitro data + experimental models)
3. Evidence for this rule is C (weak evidence)
4. The rule has been set up only to justify the job of microbiologists
5. You do not trust this rule and you never use it
**EUCAST interpretive rule 9.1 (Enterobacteriaceae)**

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# MICs of beta-lactams for selected ESBL

<table>
<thead>
<tr>
<th>ESBL</th>
<th>MIC (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cefotaxime</td>
</tr>
<tr>
<td>CTX-M-1</td>
<td>64</td>
</tr>
<tr>
<td>CTX-M-15</td>
<td>256</td>
</tr>
<tr>
<td>CTX-M-16</td>
<td>16</td>
</tr>
<tr>
<td>TEM-3</td>
<td>2</td>
</tr>
<tr>
<td>SHV-2</td>
<td>1</td>
</tr>
</tbody>
</table>

R, I, S
A murine thigh infection model for evaluation of activity of 3rd GC according to MIC

The % T>MIC was predictive of activity of 3rd/4th gen. cephalosporins against ESBL and non ESBL groups

The β-lactam MIC of an ESBL-producing isolate can be used to predict likely human outcomes from PK/PD models

Monte-Carlo simulations and target attainment rates (TAR) for intravenous ceftriaxone 2 g every 24 h

<table>
<thead>
<tr>
<th>MIC (mg/L)</th>
<th>20%</th>
<th>30%</th>
<th>40%</th>
<th>50%</th>
<th>60%</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.25</td>
<td>100</td>
<td>100</td>
<td>97</td>
<td>55</td>
<td>6</td>
</tr>
<tr>
<td>0.5</td>
<td>100</td>
<td>100</td>
<td>72</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>1.0</td>
<td>100</td>
<td>90</td>
<td>16</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2.0</td>
<td>99</td>
<td>29</td>
<td>10</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4.0</td>
<td>54</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

EUCAST Breakpoints

TAR at T>MIC (30% for ceftriaxone) for a static to one log pathogen kill at 24 h is taken to be most predictive of outcomes in humans

MacGowan A. Clin Microbiol Infect 2008; 14 (Suppl 1):166-8
Clinical failures with 3rd generation cephalosporins against ESBL producing organisms

Evidence for rule 9.1

• Weak evidence for this rule

• Both *in vitro*, experimental and clinical data rather suggest: "report as found". However, only few clinical data are available

• Are we ready to leave interpretation for ESBL?

• Revision of the rule currently under discussion
EUCAST rule 13.7 (*Salmonella* spp.)

If resistant to nalidixic acid, report as resistant to all fluoroquinolones.

1. Ciprofloxacin is an exception to the rule
2. Evidence for this rule is A for all *Salmonella*
3. Evidence for this rule is B for all *Salmonella* (*in vitro* data + experimental models)
4. Evidence for this rule is C (only in vitro data)
5. Evidence for this rule is A only for *S. Typhi*
EUCAST rule 13.7 (Salmonella spp.)

If resistant to nalidixic acid, report as resistant to all fluoroquinolones.

1. Ciprofloxacin is an exception to the rule
2. Evidence for this rule is A for all Salmonella
3. Evidence for this rule is B for all Salmonella (in vitro data + experimental models)
4. Evidence for this rule is C (only in vitro data)
5. Evidence for this rule is A only for S. Typhi (B for other salmonella)
Ciprofloxacin therapy failure against nalidixic acid-resistant *Salmonella Typhi*

- 109 patients, infected by MDR *S. Typhi*
  - Persistence of fever (>6 days) in 25 of 46 evaluable patients (54.3%).
  - 8 (17.4%) had positive blood cultures after 6 days of ciprofloxacin therapy (blood levels controlled)

- All 8 *S. Typhi* were resistant to nalidixic acid but susceptible to ciprofloxacin (MIC = 0.5 µg/mL instead of 0.032 µg/mL)

Treatment failures in patients infected with *Salmonella enterica* serovar Typhi and non-Typhi *Salmonella* isolates with decreased susceptibility to fluoroquinolones

<table>
<thead>
<tr>
<th>Country</th>
<th>Serovar</th>
<th>No. of patients</th>
<th>MIC for isolate (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Denmark</td>
<td>Enteritidis</td>
<td>1</td>
<td>Parental strain, 0.032; after initial treatment, 1</td>
</tr>
<tr>
<td></td>
<td>Typhim. DT104</td>
<td>27</td>
<td>0.064-0.124</td>
</tr>
<tr>
<td></td>
<td>Typhimurium</td>
<td>83</td>
<td>0.06-0.38</td>
</tr>
<tr>
<td></td>
<td>Typhi</td>
<td>1</td>
<td>0.19</td>
</tr>
<tr>
<td>France</td>
<td>Typhi</td>
<td>1</td>
<td>0.12</td>
</tr>
<tr>
<td>India</td>
<td>Typhi</td>
<td>32</td>
<td>0.0625-0.5</td>
</tr>
<tr>
<td>Spain</td>
<td>Enteritidis</td>
<td>2</td>
<td>Parental strains, 0.06; after treatment, 0.5 and 1</td>
</tr>
<tr>
<td>United Kingdom</td>
<td>Typhi</td>
<td>1</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>Typhimurium</td>
<td>1</td>
<td>Original strain, 0.03; after treatment, 2.0</td>
</tr>
<tr>
<td></td>
<td>Typhimurium</td>
<td>2</td>
<td>Original strains, 0.015; 0.03; after treatment, 2; 0.06-1</td>
</tr>
<tr>
<td></td>
<td>Bovismorbifica</td>
<td>1</td>
<td>Original isolate, 0.06; after treatment, 2, 16, and 4</td>
</tr>
<tr>
<td>Vietnam</td>
<td>Typhi</td>
<td>150</td>
<td>0.125-1 (ofloxacin)</td>
</tr>
</tbody>
</table>

Phenotypes of resistance to macrolides and clindamycin in *S. aureus*

- **MLS$_B$ constitutive**
  - RIBOSOMAL METHYLATION (A2058) (*erm* gene)
  - D-zone test

- **MLS$_B$ inducible**
  - EFFLUX (MsrA pump)
Clindamycin may select resistant mutants in MLS$_{Bi}$ S. aureus

Positive D-zone test: Selection of MLS$_{B}$ constitutive mutants with clindamycin [for $erm(C)$ frequency: $10^{-7}$]

Negative D-zone test: No selection of mutants with clindamycin (not substrate for the pump)
1. If D-test positive: not enough evidence for clinical failure with clindamycin therapy → report as « S » to clindamycin

2. If D-test positive, evidence for clinical failure with clindamycin therapy: grade A → report as « R » to clindamycin

3. If D-test positive, evidence for clinical failure with clindamycin therapy: grade C → report as « S » to clindamycin with a warning « Clinical failure during treatment with clindamycin may occur by selection of resistant mutants ».

4. If D-test positive, you need a molecular test to report
EUCAST rule

If D-test positive,
Either report as resistant to clindamycin and lincomycin
or report as susceptible with a warning: "Clinical failure during treatment with clindamycin or lincomycin may occur by selection of constitutively resistant mutants".
The use of clindamycin/lincomycin is probably best avoided in severe infections.
Inducibly $\text{MLS}_B$ resistant isolates: clindamycin therapy failures

<table>
<thead>
<tr>
<th>No of patients treated with clindamycin</th>
<th>No of failures</th>
<th>No of MLSB constitutive isolates selected</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>2</td>
<td>1/3</td>
<td>Rao (2000)</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>2/2</td>
<td>McGehee (1968)</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>1/3</td>
<td>Drinkovic (2002)</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>1/2</td>
<td>Frank (2001)</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>1/1</td>
<td>Siberry (2003)</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>1/1</td>
<td>Levin (2005)</td>
</tr>
<tr>
<td>12</td>
<td>9</td>
<td>7/12</td>
<td></td>
</tr>
</tbody>
</table>

Grade B
Grading of evidence base for EUCAST Expert rules

50 rules are graded A, B or C
1. 2/3 are graded C
2. 1/2 are graded A
3. 1/3 are graded A, 1/3 are graded B and 1/3 are graded C
4. 90% are graded C
Grading of evidence base for EUCAST Expert rules

- A  8 (16%)
- B  9 (18%)
- C 33 (66%, 2/3)
Conclusion

• Interpretive reading of susceptibility tests is recognized as a major process to report reliable results of AST to clinicians

• 2/3 of rules based on in vitro evidence

• Need of more clinical studies to assess the clinical impact of recommendations