Antimicrobial susceptibility testing: automatic systems

EUCAST-ESGARS Educational Workshop
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Dr. Rafael Cantón

European Society of Clinical Microbiology and Infectious Diseases
Methods for antimicrobial susceptibility testing

- **Phenotypic test methods:** based on antimicrobial activity and breakpoints
  - MIC determination (broth, agar, gradient diffusion)
  - Disk diffusion (BSAC, CA-SFM, CLSI, SRGA, EUCAST...)
  - Automated systems (Vitek, Phoenix, MicroScan, Sensititre, ...)

- **Genotypic test methods:**
  based on the detection of a resistance gene or its product
  - mecA, vanA, vanB...
  - PBP2a, β-lactamase detection

- **By deduction** – “expert rules”
  - If mecA-positive, then report beta-lactam antibiotics as R
  - If erythromycin-R, then report azithro- and clarithromycin as R
Main objectives

- To produce antimicrobial susceptibility testing (AST) results in a mechanized mode
- To standardize AST avoiding uncontrolled differences
- To offer AST in a shorter period of time than manual methods
- To interpret AST results (clinical categorization / interpretation)

a long history of more than 80 years!
Antibiosis between *Penicillium notatum* and *Staphylococcus aureus*

Fleming, 1929
The antimicrobial susceptibility testing process…

- Discover of antimicrobial agents
- Introduction of antibiotic in therapeutics
- Description of resistance mechanisms
- Relation between resistance and clinical failure
- Interpretive criteria of in vitro susceptibility testing
- Automation
- Interpretive reading of the antibiograms

Cantón R. Enf Inf Microbiol Clin 2002; 20:176-85
An early “automatic” device: the Steers’s multi-inoculator (1959) ...

Antimicrobial susceptibility automatic systems

The first “automated short-incubation system”: The TAAS device (1971)  
(Technicon Instruments Corp. Tarrytown, NY, USA)


- Bacterial growth after 3-h incubation in the presence of one antimicrobial agent concentration was compared with a 3-h control with no drug.

- The system includes:
  - inoculation unit
  - incubation unit
  - detection growth unit (optical recorder)

All these units are included in currently used automatic systems!
MIC based automatic systems
Antimicrobial susceptibility automatic systems

- **None of the current automatic susceptibility testing devices can be considered fully automated** …

  - **Automated system** consist of devices with computer-assisted incubation, reading, interpretation and reporting functions

  - **Semi-automated systems** require off-line incubation*. The panels are automatically read with computer-assisted interpretation and reporting

    *manual loading of each panel into the system is required

  - **Manual systems** use commercial (eventually in-house) panels that are read by laboratory personnel. Results are either recorded by hand or manually entered into a computer for interpretation and reporting

- **All instruments have implemented computer programs**
Antimicrobial susceptibility automatic systems

Most automatic susceptibility testing devices have incorporated ...

- Interface connections with laboratory information systems (LIS)
- Quality control computer programs
- Computer programs or expert systems:
  - to interpret phenotypes and infer resistance phenotypes
    - "antibiogram interpretive reading"
  - to perform actions based in clinical evidences and resistance mechanisms knowledge in response to specific antimicrobial susceptibility test results
    - "expert rules"
- Programs to manage results for epidemiological purpose
Antimicrobial susceptibility automatic systems

Classification

- **MIC based systems**
  - agar dilution (no longer exists!)
  - microdilution: MicroScan, Sensititre, Phoenix, …
  - growth curves: VITEK legacy, VITEK2

- **Disc diffusion based systems**
  - BIOMIC System
  - SIRSCAN System
  - OSIRIS System
  ……
## Antimicrobial susceptibility automatic systems

### MIC based systems

<table>
<thead>
<tr>
<th>Device</th>
<th>Inoculation</th>
<th>Reading</th>
<th>Reporting time (hours)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensititre</td>
<td>Manual or semiautomatic</td>
<td>Manual read or Fluorescence</td>
<td>18-24</td>
</tr>
<tr>
<td>MicroScan</td>
<td>Manual</td>
<td>Turbidity and fluorometer</td>
<td>15-18</td>
</tr>
<tr>
<td>Phoenix</td>
<td>Manual or Semiautomatic</td>
<td>Turbidity and colorimetric</td>
<td>4-16</td>
</tr>
<tr>
<td>Vitek2</td>
<td>Semiautomatic</td>
<td>Fluorometer, photometer</td>
<td>4-18</td>
</tr>
</tbody>
</table>

These systems fulfill FDA and ISO accuracy performance.

Data obtained from Evangelista & Truant. In: Commercial methods in Clinical Microbiology. 2002
Antimicrobial susceptibility automatic systems

Acceptable performance for the clinical data for automatic AST devices with reference method (FDA)

- **Essential agreement** (± 1 dilution): >89.9%
- **Category agreement** (interpretive results, SIR) >89.9%
- **Major discrepancies** (false resistance): ≤ 3%*
  *based on the no. of susceptible organisms tested
- **Very major discrepancies** (false susceptibility): ≤ 1.5%**
  **based on the no. of resistant organisms tested
- **Growth failure rates**: < 10%***
  ***for any genus or species tested

http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm080564.htm
Antimicrobial susceptibility automatic systems

Accuracy of automatic AST devices (ISO 20776-2:2007)

Data shall be analyzed by using the appropriate interpretive breakpoints

- **Essential agreement** (± 1 dilution): \( \geq 90\% \)
- **Category agreement** (interpretive results, SIR): \( \geq 90\% \)
- **Major discrepancies** (false resistance): \( \leq 3\%^* \)
  *based on the no. of susceptible organisms tested
- **Very major discrepancies** (false susceptibility): \( \leq 3\%^{**} \)
  **based on the no. of resistant organisms tested
- **Reproducibility** (± 1 dilution and/or SIR results): \( \geq 95\% \)
  ***for any genus or species tested

Main objectives

- To produce antimicrobial susceptibility testing (AST) results in a mechanized mode
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Antimicrobial susceptibility automatic systems

Vitek 2 (BioMérieux)

Time for completion of AST results (Enterobacteriaceae)

- 6.0 h → 25.9%
- 6.5 h → 53.6%
- 7.0 h → 70.3%
- 7.5 h → 81.8%
- 8.0 h → 90.5%

The utility of expert systems ...

- Computer programs associated with automatic AST systems
  - rules or algorithms using qualitative and/or quantitative results to discriminate phenotypes
  - MIC distribution data base containing all described phenotypes (matching MIC results with a database)

Antimicrobial susceptibility automatic systems

Vitek 2 (BioMérieux): Advanced expert system (AES)

Livermore et al., J Antimicrob Chemother 2002; 49: 289-300
Antimicrobial susceptibility automatic systems

... expert systems need to be updated ...

- Complex phenotypes

  ESBL + plasmid AMPc:  
  - *K. pneumoniae* 78,1% (50/64)  
  - *E. coli* 92,3% (24/26)  


- Emerging phenotypes / resistance mechanisms

  - failure to detect VIM-1 producing *K. pneumoniae* isolates
  
  - expert systems do not have the rules at the time of evaluation!

Antimicrobial susceptibility automatic systems

... most evaluations of automatic AST systems have been performed with CLSI (NCCLS) breakpoints, but ...

- Automatic systems currently available in Europe are incorporating the EUCAST breakpoints

- Among them, different systems advertise to operate on EUCAST breakpoints and/or evaluations have yet been performed:
  - MIC based systems: Phoenix, Vitek 2, MicroScan
  - Disc diffusion based systems: BioMIC
Issues with EUCAST breakpoint implementation

- Lower ranges of concentrations are needed (EUCAST breakpoints are mostly lower than CLSI)
  - instability of certain antibiotics might affect accuracy (essential and categorical agreements)
    - carbapenems, β-lactam-β-lactamase inhibitor combinations, …
  - major discrepancies (false resistance) could be observed, particularly with isolates expressing low level resistance mechanisms
Issues with EUCAST breakpoints implementation

- \( S \) and \( R \) breakpoints can be ...

  - **too close** (essential and categorical agreements can be affected)
    
    i.e. ciprofloxacin and enterobacteriaceae \((S \leq 0.5 / R > 1)\)
    
    vancomycin and staphylococci \((S \leq 2 / R > 2)\)

  - **too separate** (a higher concentration range is needed in the panel)
    
    i.e. aztreonam and \( P. \ aeruginosa \) \((S \leq 1 / R > 16)\)*

*The \( R \) breakpoint was increased from 8 to 16 mg/L to avoid dividing the wild type MIC distribution. The \( R \) breakpoint relates to high dose therapy. The \( S \) breakpoint is set to ensure that wild type isolates are reported.*
Low level resistance or decreased susceptibility

High level resistance

MIC distributions include collated data from multiple sources, geographical areas and time periods and can never be used to infer rates of resistance.

MIC (mg/L)

% microorganisms

ECOFF

17877 observations (82 data sources)
Clinical breakpoints: S ≤ 0.5 mg/L, R > 1 mg/L

Epidemiological cut-off: WT ≤ 0.032 mg/L
Aztreonam / Pseudomonas aeruginosa
EUCAST MIC Distribution - Reference Database

MIC distributions include collated data from multiple sources, geographical areas and time periods and can never be used to infer rates of resistance.

% microorganisms

MIC (mg/L)

0.002 0.004 0.008 0.016 0.032 0.064 0.125 0.25 0.5 1 2 4 8 16 32 64 128 256 ≥ 512

Epidemiological cut-off: WT ≤ 16 mg/L

Clinical breakpoints: S ≤ 1 mg/L, R > 16 mg/L

14625 observations (6 data sources)
Issues with EUCAST breakpoint implementation

- A desirable attribute...

  ... to include drug concentrations below or equal to ECOFFs* allowing detection of wild type organisms (no-R mechanism)  
  *epidemiological cut off values

- A philosophical and technical change...

  ... breakpoints are differently expressed

<table>
<thead>
<tr>
<th></th>
<th>S</th>
<th>R</th>
</tr>
</thead>
<tbody>
<tr>
<td>EUCAST</td>
<td>≤</td>
<td>&gt;</td>
</tr>
<tr>
<td>CLSI</td>
<td>≤</td>
<td>≥</td>
</tr>
</tbody>
</table>
## Antimicrobial susceptibility and automatic systems

### An example...

assessment of the Phoenix system & EUCAST breakpoints

<table>
<thead>
<tr>
<th></th>
<th>Centre A* (393 isolates)</th>
<th>Centre B** (362 isolates)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Categorical agreement</td>
<td>96.0%</td>
<td>99.1%</td>
</tr>
<tr>
<td>Interpretive discrepancies</td>
<td></td>
<td></td>
</tr>
<tr>
<td>− minor discrepancies</td>
<td>2.4%</td>
<td>2.8%</td>
</tr>
<tr>
<td>− Major discrepancies</td>
<td>1.2%</td>
<td>0.8%</td>
</tr>
<tr>
<td>− Very major discrepancies</td>
<td>1.1%</td>
<td>1.3%</td>
</tr>
</tbody>
</table>

*Morosini, García-Castillo, Cantón. Ramón y Cajal University Hospital. Madrid (Spain)

**Giani, Conte, D’Andrea, Rossoloni. University of Siena (Italy)

Posters presented at 20th ECCMID, 2010
Issues with EUCAST breakpoints implementation

- **EUCAST expert rules** must be implemented with EUCAST breakpoints and not with CLSI breakpoints!

  The case of 3rd/4th gen. cephalosporins and Enterobacteriaceae

<table>
<thead>
<tr>
<th></th>
<th><strong>CLSI (2010)</strong></th>
<th></th>
<th><strong>EUCAST (2010)</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>S</strong></td>
<td><strong>R</strong></td>
<td><strong>S</strong></td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>≤1</td>
<td>≥4</td>
<td>=</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>≤1</td>
<td>≥4</td>
<td>=</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>≤4</td>
<td>≥16</td>
<td>≤1</td>
</tr>
<tr>
<td>Cefepime</td>
<td>≤8</td>
<td>≥32</td>
<td>≤1</td>
</tr>
</tbody>
</table>
Expert rules

Cephalosporins breakpoints

<table>
<thead>
<tr>
<th>Cefalosporins</th>
<th>EUCAST</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S (≤)</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>1</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>1</td>
</tr>
<tr>
<td>Cefepime</td>
<td>1</td>
</tr>
</tbody>
</table>

There is no currently an expert rule on ESBLs as test results should be reported as found.

There may, however, be strong arguments for testing for ESBLs or other resistance mechanisms for infection control or epidemiological surveillance reasons.
Current situation in Europe

- Antimicrobial susceptibility automatic systems with EUCAST breakpoints are being implemented and introduced in Europe.

- EUCAST breakpoint implementation does not represent any fundamental problem.


- Other manufacturers are recalibrating their systems to ranges needed for EUCAST breakpoints.
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