

Implementation of the EUCAST breakpoints with automatic systems

*Antimicrobial susceptibility testing – practical implementation of the
EUCAST breakpoints and methods*

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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, MicroScans, 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”

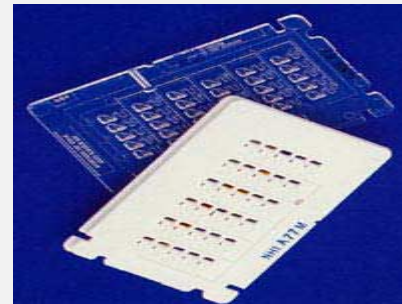
- If *mecA*-positive, then report beta-lactam antibiotics as R
- If erythromycin-R, then report azithro- and clarithromycin as R

Antimicrobial susceptibility automatic systems

Main objectives

- To produce antimicrobial susceptibility testing (AST) results in a automated (or semi-automated) 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)

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

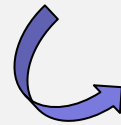
- *All instruments have implemented computer programs*

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

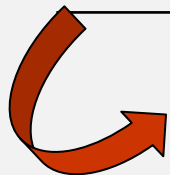
- BIOMIC System
- SIRSCAN System
- OSIRIS System

.....

Antimicrobial susceptibility automatic systems

MIC based systems

Device	Inoculation	Reading	Reporting time (hours)
Sensititre	Manual or semiautomatic	Manual read or Fluorescence	18-24
MicroScan	Manual	Turbidity and fluorometer	15-18 3.5-7
Phoenix	Manual or Semiautomatic	Turbidity and colorimetric	4-16
Vitek2	Semiautomatic	Fluorometer, photometer	4-18



These system fulfill FDA and ISO accuracy performance

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): $\leq 3\%^*$
*based on the no. of susceptible organisms tested
- **Very major discrepancies** (false susceptibility): $\leq 1.5\%^{**}$
**based on the no. of resistant organisms tested
- **Growth failure rates:** < 10%^{***}

***for any genus or species tested

Antimicrobial Susceptibility Test (AST) Systems. Guidance for Industry and FDA
Class II Special Controls Guidance Document:, August 28, 2009

<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

Clinical laboratory testing and in vitro diagnostic test systems - Susceptibility testing of infectious agents and evaluation of performance of antimicrobial susceptibility test devices - Part 2: Evaluation of performance of antimicrobial susceptibility test devices. International Standard **ISO 20776-2:2007**, ISO, Geneva.

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
- Different systems advertise that they operate with EUCAST breakpoints but evaluations have not yet been performed for all:
 - MIC based systems: Phoenix
Vitek 2
MicroScan
 - Disc diffusion based systems: BioMIC

Antimicrobial susceptibility automatic systems

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

Antimicrobial susceptibility automatic systems

Issues with EUCAST breakpoint implementation

- S and R breakpoints can be ...
 - **too close** (essential and categorical agreements can be affected)
e.g. ciprofloxacin and enterobacteriaceae ($S \leq 0.5 / R > 1$)
vancomycin and staphylococci ($S \leq 2 / R > 2$)
 - **too separate** (a wider concentration range is needed in the panel)

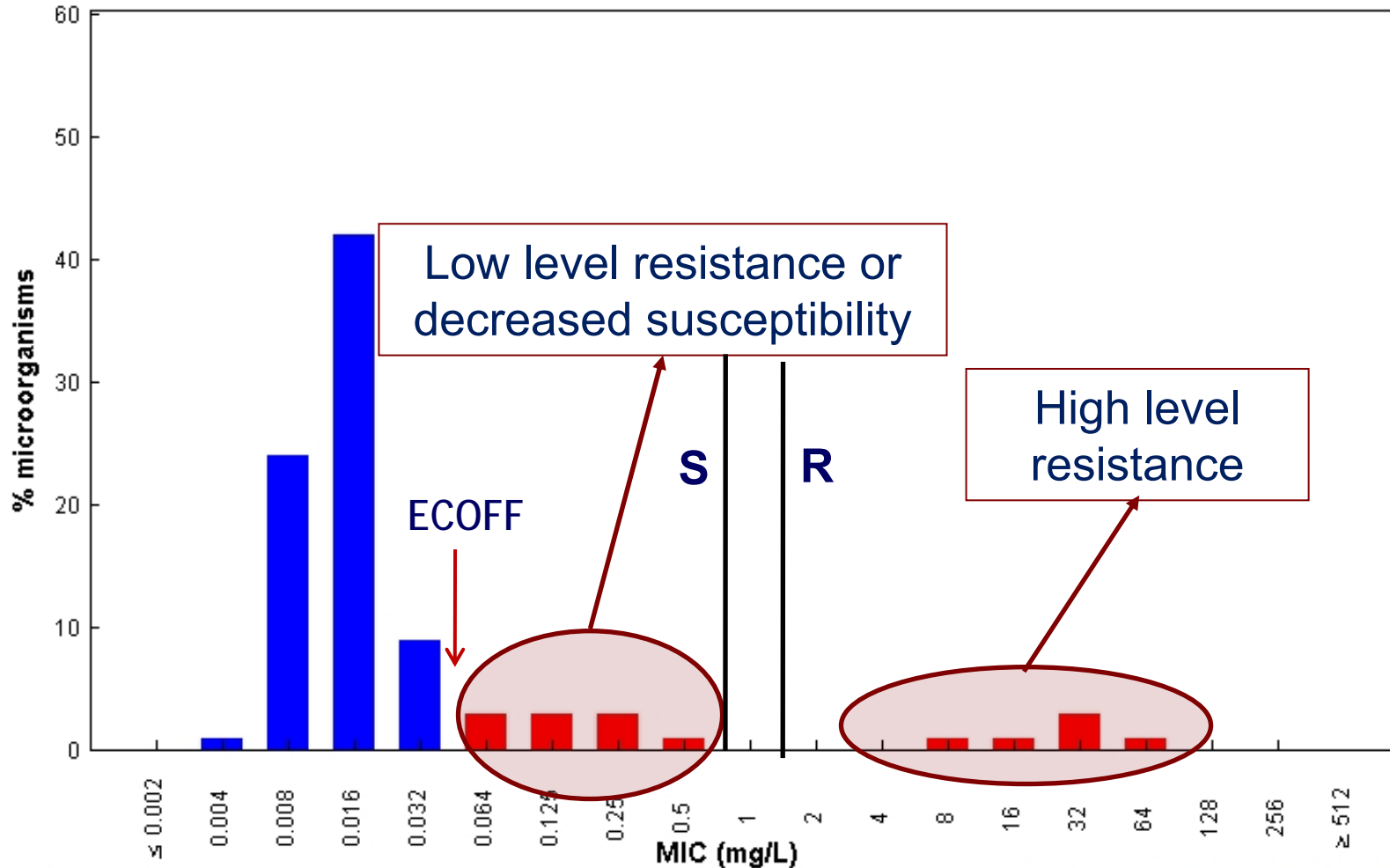
e.g. aztreonam and *P. aeruginosa* ($S \leq 1 / R > 16$)*

*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 I.

Ciprofloxacin / Escherichia coli

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



MIC

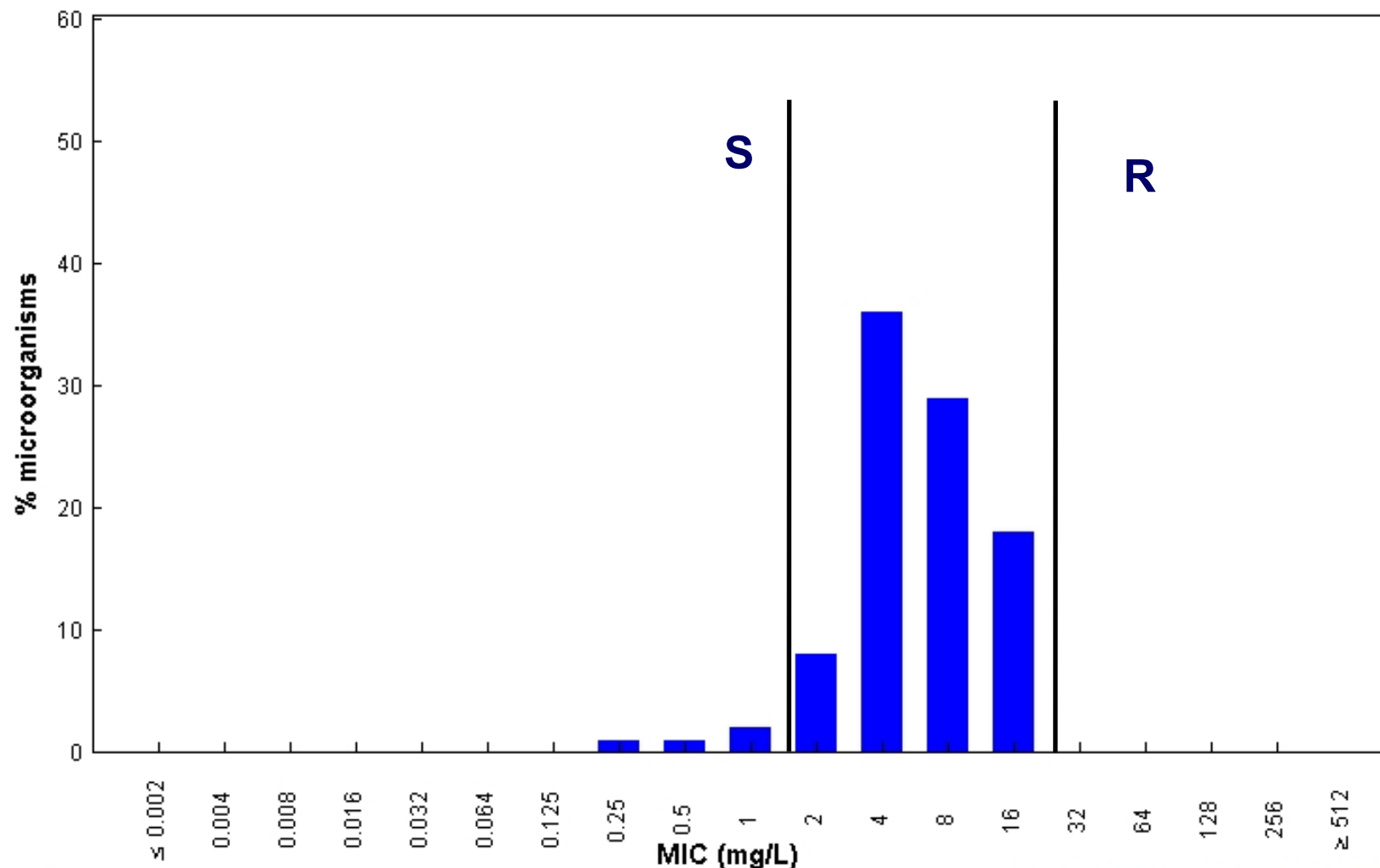
Epidemiological cut-off: WT ≤ 0.032 mg/L

17877 observations (82 data sources)

Clinical breakpoints: S ≤ 0.5 mg/L, R > 1 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



MIC

Epidemiological cut-off: WT ≤ 16 mg/L

14625 observations (6 data sources)

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

Antimicrobial susceptibility automatic systems

Issues with EUCAST breakpoint implementation

- A desirable attribute...

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

- A philosophical and technical change...

... breakpoints are interpreted and expressed differently

	S	R
EUCAST	≤	>
CLSI	≤	≥



Antimicrobial susceptibility automatic systems

An example...

assessment of the Phoenix system & EUCAST breakpoints

	Centre A* (393 isolates)	Centre B** (362 isolates)
Categorical agreement	96.0%	99.1%
Interpretive discrepancies		
- minor discrepancies	2.4%	2.8%
- Major discrepancies	1.2%	0.8%
- Very Major discrepancies	1.1%	1.3%

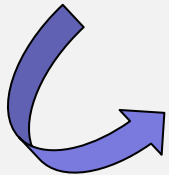
*Morosini, García-Castillo, Cantón. Ramón y Cajal University Hospital. Madrid (Spain)

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

Antimicrobial susceptibility automatic systems

Issues with EUCAST breakpoint implementation

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

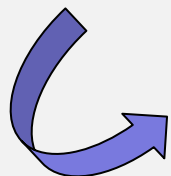


For 3rd/4th gen. cephalosporins and Enterobacteriaceae test results should be reported as found irrespective of ESBL production

	CLSI (2010)			EUCAST (2010)	
	S	R		S	R
Cefotaxime	≤1	≥4	=	≤1	>2
Ceftriaxone	≤1	≥4	=	≤1	>2
Ceftazidime	≤4	≥16		≤1	>4
Cefepime	≤8	≥32		≤1	>4

Antimicrobial susceptibility automatic systems

- Antimicrobial susceptibility automatic systems with EUCAST breakpoints are being implemented and introduced in Europe
- EUCAST breakpoint implementation does not represent any fundamental problem
- Initial evaluations of Phoenix system with EUCAST breakpoints fulfil ISO requirements (ISO 20776-2:2007).
- Other manufacturers have accepted the need for recalibrating their systems to ranges needed for EUCAST breakpoints and evaluations are being performed in different countries



Automation and EUCAST: join it!

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