REPORTING ANTIBIOGRAM DATA

Reporting MICs or Clinical Categories?

Luis Martínez-Martínez
Dept. Microbiology
University of Córdoba
Service of Microbiology
Univ. Hosp. Reina Sofía
Córdoba, Spain

Vienna, April 22 2017
Microorganisms [likely] causing an infection

Bacteria cultured from colonized patients
Identification of microorganisms to genus/species level

Definition of breakpoints (clinical BP, ECOFF)
Research
REPORTING SUSCEPTIBILITY TESTING RESULTS

Methodological issues

Conceptual issues
REPORTING SUSCEPTIBILITY TESTING RESULTS

Methodological issues
IN VITRO SUSCEPTIBILITY TESTING: METHODOLOGIES

Phenotypic assays:
Microorganism-antimicrobial agents interaction

Dilution assays: Broth dilution
  Macro dilution
  Micro dilution
  [(semi)-automated dilution devices]

Agar dilution

Diffusion assays: Paper discs/Tablets
  Gradient strips

Detection of biochemical mechanisms

Detection of resistance genes
1. MICs are the simplest (semiquantitative) estimates of the antibacterial effect in vitro. MICs are currently the reference values measuring in vitro susceptibility testing.

2. All breakpoints defining clinical categories are either
   • MICs
   • Zone diameter values correlated with MICs

[Adapted from ]Turnidge J & Paterson DL. CMR 2007, 391
REGRESSION LINE FOR TETRACYCLINE

\[ y = -2.78x + 48.49 \]

MIC-COLOURED ZONE DIAMETER HISTOGRAM TECHNIQUE

Meropenem 10 µg vs. MIC
*Pseudomonas aeruginosa*, 153 isolates
(4 data sources)

Breakpoints
- **MIC**
  - S ≤ 2, R > 8 mg/L
- **Zone diameter**
  - S ≥ 24, R < 18 mm

**ECOFF**
2 mg/L

http://www.eucast.org/ast_of_bacteria/calibration_and_validation/
“SCATTERGRAM” OF MICs vs. INHIBITION ZONE DIAMETERS
<table>
<thead>
<tr>
<th>Method</th>
<th>Standardized</th>
<th>Result</th>
<th>Clinical Categories</th>
</tr>
</thead>
<tbody>
<tr>
<td>MACRODILUTION</td>
<td>YES</td>
<td>MIC</td>
<td>YES</td>
</tr>
<tr>
<td>MICRONODILUTION</td>
<td>YES</td>
<td>MIC</td>
<td>YES</td>
</tr>
<tr>
<td>[COMMERCIAL] MICRODILUTION</td>
<td>☺</td>
<td>[MIC]*</td>
<td>YES</td>
</tr>
<tr>
<td>AGAR DILUTION</td>
<td>YES</td>
<td>MIC</td>
<td>YES</td>
</tr>
<tr>
<td>GRADIENT DIFFUSION</td>
<td>☺</td>
<td>MIC</td>
<td>YES</td>
</tr>
<tr>
<td>DISC DIFFUSION</td>
<td>YES</td>
<td>Zone diameter</td>
<td>YES</td>
</tr>
</tbody>
</table>

**DETECTION OF BIOCHEMICAL OF MECHANISMS**

- [☺]
  - Result: Pos/Neg
  - Clinical Categories: Resistant vs. not Resistant

**DETECTION OF RESISTANCE GENES**

- [☺]
  - Result: Yes/Not
  - Clinical Categories: “Resistant” vs. “Not Resistant”

*Unprecise results are obtained for some agents depending on panels/cards composition*
Slide withheld at request of author
For some organisms, only MIC assays are indicated, or clinical breakpoints have only been defined for MIC assays.
## Comment

<table>
<thead>
<tr>
<th>Drug</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mecillinam</td>
<td>AGAR DILUTION is the reference method for Mecillinam MIC determination.</td>
</tr>
<tr>
<td>Fosfomycin</td>
<td>Zone diameter breakpoints apply to <em>E. coli</em> only. For other Enterobacteriaceae, use an MIC method.</td>
</tr>
<tr>
<td>Tigecycline</td>
<td>Zone diameter breakpoints validated for <em>E. coli</em> only. For other Enterobacteriaceae, use an MIC method.</td>
</tr>
<tr>
<td>Colistin</td>
<td>Use an MIC method.</td>
</tr>
</tbody>
</table>
"The correlation between gradient tests and reference MICs was poor, even when QC results were within range. This was probably related to the poor diffusion of colistin in agar.

Based on the results of this study, EUCAST recommends laboratories to use BMD methods for colistin MIC determination and advice against the use of gradient tests at this point."

Matuschek E et al, ECCMID 2017 P161
### Pseudomonas aeruginosa

EUCAST 2017

<table>
<thead>
<tr>
<th>MIC BP (mg/L)</th>
<th>Disc Content (μg)</th>
<th>Zone diameter BP (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S&lt;=</td>
<td>R&gt;</td>
<td>S&lt;=</td>
</tr>
<tr>
<td>Ceftolozane-tazobactam</td>
<td>4</td>
<td>4</td>
</tr>
</tbody>
</table>

**CLSI MIC BREAKPOINT FOR RESISTANCE IS DIFFERENT**

([IN PRINCIPLE…] CLSI DIFFUSION BREAKPOINTS SHOULD NOT BE APPLIED IF EUCAST METHODOLOGY AND CRITERIA ARE USED IN THE LAB!!!)
Slide withheld at request of author
| DILUTION METHODS | 1. (USUALLY) BASED ON GEOMETRIC SCALE  
2. ACTUAL MIC VALUE SOMEWHERE BETWEEN THE OBTAINED MIC AND THE IMMEDIATE LOWER DILUTION  
1+2: Major impact at high MICs!!!  
3. INTRINSIC METHODOLOGICAL ERROR ACCEPTED TO BE $\pm 1$ DILUTION (or even more, depending on the organism)  
4. DIFFICULTIES FOR DETECTING CONTAMINATION IN DILUTION ASSAYS (TECHNICAL MISTAKE vs. EAGLE's EFFECT)  
5. STANDARDIZED VERSIONS ARE DIFFICULT TO IMPLEMENT IN DAILY WORK OF CLINICAL LABORATORIES |
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>GRADIENT DIFFUSION</td>
<td>1. UNRELIABLE RESULTS FOR SOME AGENTS [...NOT A STANDARDIZED METHOD!!]</td>
</tr>
</tbody>
</table>
| DISC DIFFUSION | 1. [MIC] and MBC NOT DEFINED  
2. NOT A STANDARDIZED METHOD FOR SOME AGENTS/BACTERIA |
REPORTING SUSCEPTIBILITY TESTING RESULTS

Conceptual issues
## INTERPRETATIVE CLINICAL CATEGORIES

<table>
<thead>
<tr>
<th>EUCAST</th>
<th>CLSI</th>
</tr>
</thead>
<tbody>
<tr>
<td>SUSCEPTIBLE</td>
<td>SUSCEPTIBLE</td>
</tr>
<tr>
<td>SUSCEPTIBLE-DOSE DEPENDENT</td>
<td>INTERMEDIATE</td>
</tr>
<tr>
<td>INTERMEDIATE</td>
<td>INTERMEDIATE</td>
</tr>
<tr>
<td>RESISTANT</td>
<td>RESISTANT</td>
</tr>
<tr>
<td>NONSUSCEPTIBLE</td>
<td>NONSUSCEPTIBLE</td>
</tr>
</tbody>
</table>
EUCAST: CLINICALLY SUSCEPTIBLE (S)
A microorganism is defined as susceptible by a level of antimicrobial activity associated with a high likelihood of therapeutic success. The microorganisms is categorized as susceptible by applying the appropriate breakpoint in a defined phenotypic test system.

CLSI: SUSCEPTIBLE (S)
Isolates with an MIC at or below or zone diameters at or above the “susceptible breakpoint” are inhibited by usually achievable concentrations of antimicrobial agent when the dose recommended to treat the site of infection is used, resulting in likely clinical efficacy.
**EUCAST: CLINICALLY RESISTANT (R)**
A micro-organism is defined as resistant by a level of antimicrobial activity associated with a high likelihood of therapeutic failure. A micro-organism is categorized as resistant by applying the appropriate breakpoint in a defined phenotypic test system.

**CLSI: RESISTANT (R)**
Isolates with an MIC at or above or zone diameters at or below the “resistant breakpoint” are not inhibited by usually achievable concentrations of the agent with normal dosage schedules AND/OR that demonstrate MICs that fall in the range in which specific microbial resistance mechanisms are likely, and clinical efficacy of the agent against the isolate has not been reliably shown in treatment studies.
A microorganism is defined as intermediate by a level of antimicrobial agent activity associated with uncertain therapeutic effect. It implies that an infection due to the isolate may be appropriately treated in body sites where the drugs are physically concentrated or when a high dosage of drug can be used.

It also indicates a buffer zone that should prevent small, uncontrolled, technical factors from causing major discrepancies in interpretations. [Impact on Major and Very Major errors in categorization!!!].
INTERMEDIATE (I)

- Isolates with MICs or zone diameters within the intermediate range, that approach usually attainable blood and tissue levels and for which response rates may be lower than for susceptible isolates. It implies clinical efficacy in body sites where the considered drug is physiologically concentrated or when a higher than normal dosage of a drug can be used.

- It includes a buffer zone, which should prevent small, uncontrolled technical factors from causing major discrepancies in interpretation, especially for drugs with narrow pharmacotoxicity margins.
A microorganism is defined as intermediate by a level of antimicrobial activity associated with a high likelihood of therapeutic success but only when a higher dosage of the agent than normal can be used or when the agent is physiologically concentrated at the site of infection.

A microorganism is categorized as intermediate when there is a high likelihood of therapeutic success because exposure (activity) is enhanced (1) by adjusting the dosing regimen, or (2) because the antimicrobial agent is concentrated at the site of infection.
SHOULD LABORATORIES THAT PERFORM MIC TESTS REPORT ACTUAL MIC VALUES OR RATHER PROVIDE ONLY CATEGORY INTERPRETATIONS?

…Consensus view is that in all but selected situations, only the category interpretation should be reported routinely.

This view is predicated on the relatively obscure relationship that exists between individual MIC values and defined predictions of therapeutic response together with the inherent variability of MIC determinations.
MIC values may be reported directly to clinicians for patient care purposes, AND an interpretative category results should also routinely be provided.

When disk diffusion is used, zone diameters without an interpretative category should not be reported.

It is not appropriate to apply disk diffusion or MIC breakpoints borrowed from a (breakpoint) table where the organism is not listed.
MIC IS CRITICAL FOR PD STUDIES

(A SIMILAR APPROACH WITH JUST CLINICAL CATEGORIES HAS NOT BEEN DEVELOPED)
Does resistance [always] predict failure and susceptibility predict success of antimicrobial therapy?
### “90-60” RULE

<table>
<thead>
<tr>
<th>Type(s) of infection</th>
<th>Drug(s) administered</th>
<th>Outcome measurement</th>
<th>Measurement used to determine susceptibility</th>
<th>Susceptible: 4521/5081 (89%)</th>
<th>Resistant: 215/536n(59%)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteremia and fungemia</td>
<td>Various</td>
<td>Mortality</td>
<td>MICb</td>
<td>73 (224/309)</td>
<td>48 (10/21)</td>
<td>.02</td>
</tr>
<tr>
<td>Bacteremia and fungemia</td>
<td>Various</td>
<td>Mortality</td>
<td>MICb</td>
<td>89 (594/665)</td>
<td>77 (97/126)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Serious bacterial infections</td>
<td>Various</td>
<td>Clinical response</td>
<td>MIC</td>
<td>81 (219/271)</td>
<td>4 (1/27)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Pneumococcal otitis media</td>
<td>Amoxicillin/clavulanic acid</td>
<td>Clinical response</td>
<td>MIC</td>
<td>80 (149/186)</td>
<td>68 (15/23)</td>
<td>.26</td>
</tr>
<tr>
<td>Pneumococcal otitis media</td>
<td>Cefuroxime</td>
<td>Clinical response</td>
<td>MIC</td>
<td>94 (44/47)</td>
<td>78 (29/37)</td>
<td>.05</td>
</tr>
<tr>
<td>Pneumococcal otitis media</td>
<td>Cefaclor or cefuroxime</td>
<td>Bacteriologic response</td>
<td>MIC</td>
<td>95 (55/58)</td>
<td>45 (9/20)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Pneumococcal otitis media</td>
<td>Cefaclor or azithromycin</td>
<td>Bacteriologic response</td>
<td>MIC</td>
<td>89 (23/26)</td>
<td>24 (6/25)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Bacteroides bacteremia</td>
<td>Various</td>
<td>Bacteriologic response</td>
<td>MIC</td>
<td>88 (60/68)</td>
<td>57 (4/7)</td>
<td>.06</td>
</tr>
<tr>
<td>Moderate-to-severe bacterial infections</td>
<td>Ciprofloxacin</td>
<td>Bacteriologic response</td>
<td>AUC/MIC ratio</td>
<td>82 (37/45)</td>
<td>26 (5/19)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Bacterial infections</td>
<td>Aminoglycosides</td>
<td>Clinical response</td>
<td>Peak/MIC ratio</td>
<td>~90d</td>
<td>~55d</td>
<td></td>
</tr>
<tr>
<td>Bacterial infections</td>
<td>Cefotaxime</td>
<td>Bacteriologic response</td>
<td>Zone diameter</td>
<td>92 (1464/1591)</td>
<td>63 (31/49)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Bacterial infections</td>
<td>Ciprofloxacin</td>
<td>Bacteriologic response</td>
<td>Zone diameter</td>
<td>91 (1652/1815)</td>
<td>62 (8/13)</td>
<td>.004</td>
</tr>
<tr>
<td><strong>Successful outcome</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Susceptible: 4521/5081 (89%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Resistant: 215/536n(59%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Rex JH, Pfaller MA. CID, 2002:982
For susceptible organism, is clinical response better for those with lower MICs?
# Outcome and MICs in Patients with Infection Treated with Cefotaxime (1983!)

<table>
<thead>
<tr>
<th>Cefotaxime MIC (mg/L)</th>
<th>CAT</th>
<th>Number of Patients</th>
<th>% Cured or Improved</th>
<th>% Erradication</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;=4</td>
<td>S</td>
<td>1003</td>
<td>94</td>
<td>91</td>
</tr>
<tr>
<td>8</td>
<td>S</td>
<td>273</td>
<td>90</td>
<td>86</td>
</tr>
<tr>
<td>16</td>
<td>I</td>
<td>151</td>
<td>77</td>
<td>75</td>
</tr>
<tr>
<td>32</td>
<td>I</td>
<td>70</td>
<td>84</td>
<td>71</td>
</tr>
<tr>
<td>&gt;=64</td>
<td>R</td>
<td>19</td>
<td>64</td>
<td>50</td>
</tr>
</tbody>
</table>

EUCAST BP Cefotaxime-Enterobacteria (2017): ≤1 (S); >2 (R)

Murray P et al. 1983, 23rd ICAAC, abst. 545
[Doern GV & Brecher SM, JCM 2011:S11]
OUTCOMES OF PATIENTS WITH BACTEREMIA BY *K. pneumoniae* PRODUCING KPC-2/KPC-3 TREATED WITH MEROPENEM COMBINATIONS

<table>
<thead>
<tr>
<th>MEROPENEM MIC (mg/L)*</th>
<th>Survivors %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>100 (1/1)</td>
</tr>
<tr>
<td>2</td>
<td>100 (4/4)</td>
</tr>
<tr>
<td>4</td>
<td>80 (8/10)</td>
</tr>
<tr>
<td>8</td>
<td>75 (3/4)</td>
</tr>
<tr>
<td>&gt;=16</td>
<td>64.7 (11/17)</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>75 (27/36)</strong></td>
</tr>
</tbody>
</table>

*MIC determined with Vitek2*
EUCAST 2017

- The carbapenem BP for Enterobacteriaceae will detect all clinically important resistance mechanisms (including most of carbapenemases).
- Carbapenemase-producing isolates should be reported as tested.
- Carbapenemase detection and characterisation recommended for public health and infection control purposes.

CLSI 2017

- MIC breakpoints in M100-S20 (January 2010): Perform the MHT, the Carba NP test, mCIM, and/or a molecular assay when [...] imipenem or meropenem MICs of 2–4 μg/mL or ertapenem MIC of 2 μg/mL
- After implementation of the current breakpoints, these additional tests do not need to be performed other than for epidemiological or infection control purposes.
DETECCIÓN OF RESISTANCE MECHANISMS.
CARBAPENEMASES (2017).

Disk diffusion

Commercial Panels?
PATIENTS WITH MRSA BACTEREMIA

<table>
<thead>
<tr>
<th>VANCOMYCIN MIC AND MORTALITY</th>
<th>VANCOMYCIN MIC (mg/L)*</th>
<th>OR (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.5</td>
<td>2.86 (0.87-9.35)</td>
<td>0.08</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>6.39 (1.68-24.3)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>VANCOMYCIN MIC AND SHOCK</th>
<th>VANCOMYCIN MIC (mg/L)*</th>
<th>OR (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.5</td>
<td>0.59 (0.33-1.05)</td>
<td>0.07</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.33 (0.15-0.75)</td>
<td>0.012</td>
</tr>
</tbody>
</table>

*MIC determined with Etest®

Soriano A GL et al. CID, 2008:193
“No significant differences in risk death were observed in subgroups with high-vancomycin MIC vs low-vancomycin MIC values across different study designs, microbiological susceptibility assays, MIC cutoffs, clinical outcomes, duration of bacteremia, previous vancomycin exposure, and treatment with vancomycin”

VANCOMYCIN MICs: ≥1.5mg/L vs. <1.5mg/L

EUCAST VANCOMYCIN BREAKPOINTS FOR S. aureus: ≤2 (S) ; >2 (R)
No mortality

Other source
N = 28

Low MIC
Mortality: 0/2

Intermediate MIC
Mortality: 3/8 (37.5%)c

High MIC
Mortality: 4/9 (44.4%)d

Lower mortality among patients with low-MIC isolates
## URINARY CONCENTRATIONS OF CIPROFLOXACIN AFTER ORAL ADMINISTRATION

<table>
<thead>
<tr>
<th></th>
<th>Urinary Cmax (mg/L)</th>
<th>Urinary through Conc. (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ciprofloxacin 500 mg</td>
<td>268 (130–967)</td>
<td>13 (5.1–37)</td>
</tr>
<tr>
<td>Ciprofloxacin 1000 mg</td>
<td>892.52 ± 476.4</td>
<td>32.80 ± 22.01</td>
</tr>
</tbody>
</table>

## Clinical Breakpoints Enterobacteria

<table>
<thead>
<tr>
<th></th>
<th>EUCAST</th>
<th>CLSI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ciprofloxacin</td>
<td>0.25-0.5</td>
<td>1-[2]-4</td>
</tr>
</tbody>
</table>
PATIENTS WITH BACTEREMIA CAUSED BY ENTEROBACTERIACEAE TREATED WITH PIP-TZB

EUCAST BREAKPOINTS FOR PIP/TZB: ≤8 (S), 16 (I), >16 (R)

A borderline (S/I) MIC (8-16 mg/L) of PIP/TZB was NOT associated with a worse outcome than a lower (S) MIC (<1-1-2-4 mg/L)

IF the organism is SUSCEPTIBLE, the actual MIC of PIP/TZ has no significant impact on clinical outcome

Delgado-Valverde M et al. JAC, 2016:521
Detection of resistance mechanisms
Low vs. high level resistance
Emerging mechanisms
Screening cut-off MIC values

Interpretative reading of antibiograms

Microorganisms identification
(intrinsic resistance)
MICs FOR DEFINING BREAKPOINTS

Ciprofloxacin / Escherichia coli
International MIC Distribution - Reference Database 2017-04-21

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 (ECOFF): 0.064 mg/L
Wildtype (WT) organisms: ≤ 0.064 mg/L

16702 observations (55 data sources)
EME-Central registration procedure

The company will provide:

- Proposed indications for the agent
- Proposed dosing regimens for the agent (by indication) and the available formulations
- Proposed target organisms
- MIC distributions for relevant species
- Pharmacokinetic data
- Pharmacodynamic data
- Modelling data, such as Monte Carlo simulations
- Clinical trial data, including outcome related to MIC where available

http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/EUCAST_SOPs/EUCAST_SOP_1.2_Setting_breakpoints_new_agents_20161121.pdf
CLINICAL CATEGORIES SHOULD BE ALWAYS REPORTED

IF MIC HAS BEEN OBTAINED, IT WOULD BE PREFERABLE ALSO REPORTING IT, NOT ONLY FOR BEING USEFUL TO (MANY) CLINICIANS, BUT ALSO BECAUSE OF ADEQUATE INFORMATION IN CLINICAL CHARTS

CONTINUING EDUCATIONAL ACTIVITIES EXPLAINING THE ACTUAL MEANING, ADVANTAGES AND SHORTCOMINGS OF ANTIBIOGRAM METHODS, MICs AND CLINICAL CATEGORIES SHOULD BE CONSIDERED