**In Vitro Susceptibility Results and In Vivo Efficacy**

*Po-Ren Hsueh*
National Taiwan University Hospital

**In Vitro susceptibility Results**

**Clinical Application**
- A drug lacking laboratory evidence of activity is unlikely ever to reach clinical application
- Hospital-wide implications
  - Decision of treatment of individual patient
  - Development of antibiotic formularies
  - Application of infection control policies
- In vitro susceptibility results predicting therapeutic outcome?

**MIC Determination**

- Etest
- MicroScan
- Phoenix
- Vitek 2

**MIC Breakpoints**

**How to Define**
- Epidemiological data
- Pharmacological considerations (PK/PD)
  - Cmax, Cmax/MIC, AUC/MIC, T>MIC, Cmin, T1/2
- Clinical evaluation
- Post market surveillance

**Susceptible (S)**

**Definition**

*An infection due to the strain may be appropriately treated with the dosage of antimicrobial agent recommended for that type of infection and infecting species, unless otherwise contraindicated*

**Resistant (R)**

**Definition**

- Strains are not inhibited by the usually achievable systemic concentration of the agent with normal dosage schedules
- Strains with MIC fall in the range where specific microbial resistance mechanisms are likely and clinical efficacy has not been reliable in treatment studies
**Intermediate (I)**

**Definition**
- Isolates with MICs approaching usually attainable blood and tissue levels.
- Response rates may be lower than for susceptible isolates.
- Clinical applicability in infected sites where the drugs are physiologically concentrated or high dosage of a drug can be used.
- “Buffer zone”

---

**Cefoperazone for GNB Infection (N=276)**

Clinical Outcome vs. MICs

<table>
<thead>
<tr>
<th>MIC (µg/mL)</th>
<th>% of clinical failure</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤1</td>
<td>4</td>
</tr>
<tr>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td>32</td>
<td>32</td>
</tr>
<tr>
<td>≥64 (clinical success)</td>
<td>56</td>
</tr>
</tbody>
</table>

Cefoperazone: S, ≤16 µg/mL; I, 32 µg/mL; R, ≥64 µg/mL

---

**Breakpoints Associated with Clinically-relevant Outcomes**

- **Susceptible**
- **Resistant**

---

**Breakpoint Associated with Clinically-relevant Outcomes**

- **Susceptible**
- **Resistant**

---

In vitro susceptibility and in vivo efficacy

---

In vitro resistance and in vivo efficacy

---

In vitro susceptibility and in vivo resistance
In Vitro Susceptibility and In Vivo Resistance (Treatment Failure)

Factors Influencing Correlations between In Vitro Susceptibility Results and Effectiveness

- Agents, pathogens, and hosts
- In vitro testing
  - Standard conditions (constant phase of bacterial growth, fixed temperature, humidity, and O2)
  - Exposure of a small number of organisms (10^5-10^6 CFU) to constant levels of agents (heteroresistant)
  - Breakpoints selected for bacteremia (serum conc.)
  - Inhibitory activity
- In patients
  - Nothing is standardized or static
  - Large number (>10^9) to fluctuating levels of agents
  - Deep-seated (less penetration), need bactericidal activity

Factors Influencing Correlations between In Vitro Susceptibility Tests and Effectiveness

Antimicrobial Agents

| Pharmacodynamics | Inhibitory activity, bactericidal activity, concentration-dependent, time-dependent, "Eagle effect", post-antibiotic effect |
| Pharmacokinetics  | Absorption, distribution, protein binding, metabolism, excretion, intracellular concentration, penetration |
| Microbial resistance mechanisms | Intrinsic resistance, Acquired resistance, Secondary resistance (co-resistant) |

Pharmacodynamics

- Inhibitory activity, bactericidal activity, concentration-dependent, time-dependent, "Eagle effect", post-antibiotic effect
- Absorption, distribution, protein binding, metabolism, excretion, intracellular concentration, penetration
- Intrinsic resistance, Acquired resistance, Secondary resistance (co-resistant)

Pharmacokinetics

- Inhibitory activity, bactericidal activity, concentration-dependent, time-dependent, "Eagle effect", post-antibiotic effect
- Absorption, distribution, protein binding, metabolism, excretion, intracellular concentration, penetration
- Intrinsic resistance, Acquired resistance, Secondary resistance (co-resistant)

Microbial resistance mechanisms

- Intrinsic resistance
- Acquired resistance
- Secondary resistance (co-resistant)

Factors Influencing Correlations between In Vitro susceptibility Tests and Effectiveness

Hosts

| Specific and systemic | Complement activation, Cellular immunity, Humoral immunity |
| Nonspecific and systemic | Chemotaxis, phagocytosis, and killing, Natural killer cell functions, Cytokines response, Inflammation, Comorbid condition |
| Site of infection | Site-specific effectiveness of immune response, Abscess/sequestration formation, Presence of foreign bodies, PH, Anaerobiosis |

Penicillin Resistance in S. pneumoniae

2008 Definition of Penicillin Susceptibility

<table>
<thead>
<tr>
<th>Category</th>
<th>Non-meningitis (µg/mL)</th>
<th>Meningitis (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Previous</td>
<td>2008*</td>
</tr>
<tr>
<td>Susceptible</td>
<td>≤ 0.06</td>
<td>≤ 0.06</td>
</tr>
<tr>
<td>Intermediate</td>
<td>0.12 - 1</td>
<td>4</td>
</tr>
<tr>
<td>Resistant</td>
<td>≥ 2</td>
<td>≥ 2</td>
</tr>
</tbody>
</table>

Tigecycline

FDA-Approved Interpretive Criteria

<table>
<thead>
<tr>
<th>Organism</th>
<th>MIC (µg/mL)</th>
<th>Disk diffusion (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S</td>
<td>I</td>
</tr>
<tr>
<td>S. aureus</td>
<td>≤0.06</td>
<td>≤0.06</td>
</tr>
<tr>
<td>Streptococcus spp. (other than S. pneumoniae)</td>
<td>≤0.06</td>
<td>≤0.06</td>
</tr>
<tr>
<td>Enterobacteriaceae</td>
<td>≤2</td>
<td>4</td>
</tr>
<tr>
<td>Acinetobacter spp. a</td>
<td>≤2</td>
<td>4</td>
</tr>
<tr>
<td>Anaerobes (agar dilution)</td>
<td>≤4</td>
<td>8</td>
</tr>
</tbody>
</table>

*Tigecycline has decreased in vitro susceptibility against Morganella spp., Proteus spp., and Providencia spp.

M100-S21 CLSI 2011

Higher Infection-related Mortality in IVDU with MSSA IE

Vancomycin vs β-lactams

- MICs of both agents: similar
- Vancomycin: delayed response, prolonged bacteremia, and fever
- β-lactams: more rapidly and completely bactericidal than vancomycin

IVDU, intravenous drug users


Nosocomial MRSA Bacteremia
Mortality based on Vancomycin MICs

Failure of Current Cefepime Breakpoints To Predict Clinical Outcomes of GNB Bacteremia
MICs vs. Outcome

Persistent MRSA Bacteraemia (≥7 days) in Patients Treated with Vancomycin
Predictors- Multivariate Analysis

Risk factorsa OR P value
Vancomycin MIC of 2 mg/L 6.34 0.029
Retention of implicated medical devices 10.35 0.048
MRSA infection of at least two sites 10.24 0.011


30-Day Mortality Rate for P. aeruginosa Bacteremia
Based on Piperacillin-tazobactam MIC

Susceptible CLSI: ≤64 μg/mL; EUCAST: ≤16 μg/mL

Rapidly Fatal
Toxin-related Infections
Died within 48 hrs due to Susceptible Strains

S. aureus (MSSA)  V. vulnificus  S. pyogenes
Challenges in Interpretation of Antifungal Susceptibility Results

- MIC values do not always directly associate with response to antifungal therapy
- “90–60 rule”
  - Infections caused by susceptible strains respond to appropriate therapy in 90% of cases
  - Infections caused by resistant strains respond in 60% of cases
- Early treatment of fungal infection with a lower burden of organisms reduces the number of treatment failures


Challenges in Interpretation of Antifungal Susceptibility Results

Factors
- Treatment failure and poor outcome
  - Negative host status, delay of early diagnosis and a lack of adequate antifungal therapy
  - Toxicities from polyenes and azoles
  - Drug–drug interactions
  - Biofilms on foreign bodies
  - Immune reconstitution inflammatory syndrome


Penicillin Susceptibility among S. pneumoniae

Meningitis Criteria, PSSP, PISP, PRSP - CARTIPS-2009

- Non-meningitis criteria: Nonsusceptible rate
  - China: 10.8%; Taiwan: 2.8%; Singapore: 4%

In Vitro Susceptibility and In Vivo Resistance (Treatment Failure)

In Vitro Resistance and In Vivo Efficacy

MIC Breakpoints for FQs

Enterobacteriaceae

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Susceptible</th>
<th>Intermediate</th>
<th>Resistant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ciprofloxacin</td>
<td>≤1</td>
<td>≤0.5</td>
<td>≥4</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>≤2</td>
<td>≤1.0</td>
<td>≥8</td>
</tr>
<tr>
<td>Lomefloxacin (urine)</td>
<td>≤2</td>
<td>4</td>
<td>≥8</td>
</tr>
<tr>
<td>Ofloxacin (urine)</td>
<td>≤2</td>
<td>4</td>
<td>≥8</td>
</tr>
<tr>
<td>Norfloxacin (urine)</td>
<td>≤4</td>
<td>8</td>
<td>≥16</td>
</tr>
</tbody>
</table>

CLSI 2011 M100 S21

Pharmacokinetic Profile of FQ in UTI

Urinary Bactericidal Titers (UBTs) Levofloxacin, *E. coli*, 750 mg Single Dose

![Graph showing UBT titers](image)

New MIC Breakpoints for Uropathogens
Selected Fluoroquinolones

- A clinical trial of levofloxacin (750 mg/d) vs. ciprofloxacin (500 mg twice daily) for acute pyelonephritis, four patients had FQ-resistant isolates of *E. coli*.
  - Three were eradicated by the study drug
  - One with levofloxacin (MIC of 32 µg/mL) and two with ciprofloxacin (8 µg/mL and >32 µg/mL)
- A scenario for 750 mg of levofloxacin would be a susceptibility MIC breakpoint in urine between 16 and 32 µg/mL (UBT up to MIC of 32 µg/mL)
  - Urosepsis or tissue infections (cUTI)?

Bacteremia due to CRAB
55 Patients, NTUH, 2003-2004

![Graph showing mortality](image)

Carbapenem + Sulbactam Might Fail in CRAB with Higher MICs

- MIC of carbapenem: 256 µg/mL sulbactam: 128 µg/mL
- Carbapenem alone: MIC = 64 µg/mL sulbactam: 16 µg/mL

Imipenem in VAP
Pharmacokinetics, % T > 4xMIC

- In conclusion: a 2 h infusion of 1 g for every 6 h should be used to treat infections caused by pathogens with an MIC of 4 mg/L

Antifungal Therapy for Candidiasis
“90:60” Rule

- 90% of susceptible isolates respond to therapy
- 60% of resistant isolates respond to therapy

### Relationship between MIC and Outcome for Invasive Candidiasis with Micafungin

**Revised CLSI R-BP (2011)**

- % of successively treated
- No. of Patients successfully treated:
  - 0.016 (13/1697)
  - 0.03 (116/152)
  - 0.065 (13/13)
  - 0.125 (12/14)
  - 0.25 (15/17)
  - 1 (28/31)
  - 2 (5/9)
  - 2 (327/410)
- albicans tropicalis

### Macrolide-Resistant M. pneumoniae

- **Mutations in 23S rRNA gene**
- **Resistance (clarithromycin, azithromycin MICs >128 µg/mL)**
  - Japan: 20% of specimens collected from pediatrics (83% (44/53) and 90% (90/100))
  - A corresponding increase has not been seen in the number of clinical treatment failures (IDSA)
  - High intracellular concentration
  - Additional immunomodulating effects?
  - In vitro susceptibility testing is not available for daily management

**Additional immunomodulating effects?**
- **Topical and local administration**
- **Resistance**
- **High intracellular concentration**
- **Combination and prolonged infusion therapy**
- Infection sites that the drugs are physiologically
  - A corresponding increase has not been seen in the
- Don’t choose an agent with in vitro resistance if other susceptible drugs are available

### In Vitro Resistance and In Vivo Efficacy

- Immunocompetent vs. immunocompromised
- Different MIC breakpoints used
- Infection sites that the drugs are physiologically concentrated (UTI) or with higher dose
- Combination and prolonged infusion therapy
- Intracellular pathogens
- Topical and local administration
- Inhaled therapy (colistin...)

**In conclusion:**
- The MR patients showed more febrile days (by a median of 2 days) during the initial macrolide therapy than MS patients.
- No apparent treatment failure or serious illness was reported for MR patients.
- The influence of the emergence of MR M. pneumoniae on the treatment for M. pneumoniae infection deserves further study.

**Clinical Evaluation**

**Macrolide-Resistant M. pneumoniae**

- MR patients (n=11)
- MS patients (n=26)

<table>
<thead>
<tr>
<th>% of patients</th>
<th>Days</th>
<th>Febrile days (median)</th>
<th>Febrile days (median) during macrolide administration</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>0.0</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>5</td>
<td>2.0</td>
<td></td>
<td>1</td>
</tr>
</tbody>
</table>