Panel of: Antimicrobial sensitivity tests, control and prevention

Performance of Standards for Antimicrobial Susceptibility Testing (AST)

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Introduction

- Heavily information from the microbiology laboratory causes challenge for clinicians.
- Over time, a microorganism’s susceptibility to an antimicrobial agent may decrease, resulting in a lack of clinical efficacy and/or safety.
- In addition, standard microbiological methods and QC parameters may be refined to ensure more accurate and better performance of antibiotic susceptibility test methods (CLSI).
Cont’d

- Infections need to be treated quickly to keep them from spreading, especially in critical cases such as blood infections.
- But bacteria are constantly mutating and achieving resistance to antibiotics, making it harder for medical personnel to respond in time with the right drugs.
Empirical therapy, is used when:

- No enough time to AST.

- This means probable antibiotics resistance is assessed on the basis of the population the patient belongs to – for instance a country's general public.

- The next tactic is: to treat the patient with broad spectrum antibiotics.

- But these are less effective than targeted antibiotics and their use can contribute to antibiotic resistance.
STANDARDIZATION

- For laboratory tests to accurately determine organism based resistances, the potential influence of environmental factors on antibiotic activity should be minimized. So, need to:
  - Optimizes bacterial growth conditions
  - Optimizes conditions for maintaining antimicrobial integrity and activity
  - Maintains reproducibility and consistency in the resistance profile of an organism
Cont’d

- **Early and accurate** recognition of resistant bacteria significantly aids the **selection of antimicrobial therapy and optimal patient management**. Thus:

- In vitro AST provides valuable data that are used in conjunction with other diagnostic information to **guide patient therapeutic options**.
Direct measures of antimicrobial activity are accomplished using:

- **Conventional susceptibility testing methods** such as: *dilution* (broth, agar), and *disk diffusion*.

- **Commercial susceptibility testing systems** (E-TEST, Automated methods).

- **Special screens and indicator tests** *(CAT reagent kit)*
Standard methods for antimicrobial sensitivity testing

- **AST methods**: are in vitro procedures used to detect antimicrobial resistance in individual bacterial isolates.

- **Clinical Breakpoints** are threshold values established for each pathogen-antibiotic combination indicating at what level of Ab against the isolate should be considered to use S, I or R.

- The interpretative criteria for these are based on extensive studies that correlate laboratory resistance data with serum achievable levels for each antimicrobial agent and a history of successful and unsuccessful therapeutic outcomes.
Standard conditions for these assays have been established based on extensive batteries of laboratory testing.

Guidelines and recommendations for these are continuously updated by certain organizations worldwide, such as CLSI, EUCAST, OIE (is reference organization for standards relating to animal health and zoonosis), BSAC (British Society For Antimicrobial Chemotherapy), SFM (California Referenced Standards Code), SRGA (the Swedish Reference Group for Antibiotics) and Australia.
Lab approaches and strategies

Some points to consider when deciding whether or not to conduct an AST:

- Clinical relevance of the isolate
- Purity of the isolate
- Logical panel of antimicrobial agents to be tested (i.e., do not include antibiotics to which the isolate is known to have intrinsic resistance)
- Availability of test methodology, resources, and trained personnel
- Standardization of testing
- Valid interpretation of results
- Cost efficiency
- Effective means to communicate results and interpretation to end-users.
Aspects of quality control

- One critical aspect is following standardized procedures that can generate reproducible results, i.e. quality control.

- QC includes:
  - Standardized bacterial inoculum size culture (St 0.5McFarland; 1.5x10^8 CFU/mL)
  - Conditions (growth medium, pH, cation concentration)
  - Blood and serum supplements and thymidine content
  - Incubation conditions (atmosphere, temperature, duration)
  - Concentration of antimicrobials for testing.
  - Site of Infection (e.g.; NTF and UT not other sites)
Test Methods in Detecting Antimicrobial Resistance

- **Dilution method;** broth (Agar/Broth) and (Macrodilution /Microdilution)
- **Disk-diffusion method** (Kirby-Bauer method)
- **E-test** strip method
- **Automated**
- **Resistant Mechanism-specific tests:** (e.g: β-lactamase detection test and chromogenic cephalosporin test)
- **Genotypic methods:** such as PCR and DNA hybridization methods
MIC

MIC, values determined as described in M07-A10 may be reported directly to clinicians for **patient care purposes**.

The MIC obtained using a dilution test may tell a physician the concentration of antimicrobial agent required at the site of infection to inhibit the infecting organism. However, the MIC does not represent an **absolute** value.

It is essential that an **interpretive category** result (S, I, or R) also be provided routinely to facilitate understanding of the MIC report by clinicians.

In place of interpretive criteria (“breakpoints” or “clinical breakpoints”) an epidemiological cutoff value (ECV) may be listed for specific organism/antimicrobial agent combinations.
1- Dilution methods

MIC determination

- Agar dilution
- Micro broth dilution (0.05-0.1ml)
- Macro broth dilution (1ml)
Notes

- The Ab concentration range may be based on; the level of drug required to reliably detect a particular resistance mechanism.
- In this case, the test concentration for a drug may vary depending on the organism and its associated resistances.
- For example, to detect clinically significant resistance to cefotaxime in *S. pneumoniae*, the dilution scheme uses a maximum concentration of 2 μg/mL; however, to detect cefotaxime resistance in *E. coli*, the required maximum concentration is 16 μg/mL or higher.
Inoculation in microdilution

- If the volume of broth in the well is 0.1 mL and the inoculum volume is 0.01 mL, then the 0.5 McFarland suspension (1 \( \times \) \( 10^8 \) CFU/mL) should be diluted 1:20 to yield \( 5 \times 10^6 \) CFU/mL.

- When 0.01 mL of this suspension is inoculated into the broth, the final test concentration of bacteria is approximately \( 5 \times 10^5 \) CFU/mL (or \( 5 \times 10^4 \) CFU/well in the microdilution method).
Inoculation in macrodilution

- Diluting the 0.5 McFarland suspension 1:150, resulting in a tube containing approximately $1 \times 10^6$ CFU/mL.
- The subsequent 1:2 dilution in step 3 brings the final inoculum to $5 \times 10^5$ CFU/mL.
A. Bacterial growth profiles in a broth microdilution tray. The wells containing the lowest concentration of an antibiotic that completely inhibits visible growth (arrow) are recorded in micrograms per milliliter (µg/mL) as (MIC).
2- Disk diffusion method

- Because of convenience, efficiency and cost, the disk diffusion method is probably the most widely used method for determining antimicrobial resistance method. Included of:
  - Mueller-Hinton agar (MHA)
  - Bacterial lawn of inoculation: approximately 1 to 2 x $10^8$ CFUs/ml
  - Zone diameter of inhibition
  - Results: S, I, R

Note: MIC measurement cannot be determined from this qualitative test.
A, Disk diffusion method: antibiotic disks are placed on the agar surface just after inoculation of the surface with the test organism. B, Zones of growth inhibition around various disks are apparent after 16 to 18 hours of incubation.
Reading and Interpretation of Results

- The plate is examined to confirm that a confluent lawn of growth has been obtained.
- If growth between inhibitory zones around each disk is poor and nonconfluent, the test should not be interpreted and should be repeated.
- The lack of confluent growth may be due to insufficient inoculum.
- Alternatively, a particular isolate may have undergone mutation, and growth factors supplied by the standard medium are no longer sufficient to support robust growth.
- In the latter case, medium supplemented with blood and/or incubation in CO₂ may enhance growth.
- Plates should also be examined for purity.
Instructions for Use of Tables

- Tables 1A and 1B in CLSI —Suggested groupings of antimicrobial agents that should be considered for routine testing and reporting by clinical microbiology laboratories.

- For each organism group, an additional table (Tables 2A through 2I) contains:
Selecting Antimicrobial Agents for Testing and Reporting

- Selection of the most appropriate antimicrobial agents to test and to report is based on the:
- A decision best made by each laboratory in consultation with the infectious diseases practitioners and the pharmacy.
- The recommendations for each organism group include agents of proven efficacy.
Drugs listed together in a single box are agents for which interpretive results (S, I, or R) and clinical efficacy are similar.

Within each box, an “or” between agents indicates those agents for which cross-resistance and cross-susceptibility are nearly complete. EX: cefotaxime S = ceftriaxon S
Test/Report Groups

- Agents in **Group A**: are appropriate for inclusion in a routine, primary testing panel, as well as for routine.
- Agents in **Group B**: are may warrant primary testing, but they may be reported only selectively, such as when the organism is resistant to agents of the same antimicrobial class, as in Group A.

- Selected specimen source (eg, a 3th-generation cephalosporin for enteric bacilli from CSF or SXT for UT isolates);
- A polymicrobial infection,
- Infections involving multiple sites,
- Cases of patient allergy or intolerance,
- Failure to respond to an antimicrobial agent in Group A;
- For purposes of infection control.
Cont’d

- **Group C;** includes alternative or supplemental antimicrobial agents that may necessitate testing in those institutions that harbor endemic or epidemic strains resistant to several of the primary drugs (especially in the same class, eg, β-lactams);
- Treatment of patients allergic to primary drugs
- Treatment of unusual organisms (eg; chloramphenicol for extra-intestinal isolates of *Salmonella* spp).
Cont’d

- **Group U (“urine”),** includes certain antimicrobial agents (eg, NTF and certain quinolones) that are used only or primarily for treating urinary tract infections (UTIs).

- These agents should not be routinely reported against pathogens recovered from other sites.
Cont’d

- **Group O ("other")** includes agents with clinical indication for the organism group but are generally not candidates for routine testing and reporting.

- **Group Inv. ("investigational")** includes antimicrobial agents that are investigational for the organism group and have not yet been approved by the FDA.
Reporting Results

✓ It is essential that an interpretive category result (S, I, or R) also be provided routinely to facilitate understanding of the MIC report and zone diameter by clinicians.

✓ Zone diameter measurements without an interpretive category should not be reported.
Susceptible (S) Dose

- Implies that isolates are inhibited by the usually achievable concentrations of antimicrobial agent.

- **DDS; NOTE:** The SDD interpretation is a new category for antibacterial susceptibility testing, although it has been previously applied for interpretation of antifungal susceptibility test results.

- Implies that susceptibility of an isolate is dependent on the dosing regimen that is used in the patient. The concept of SDD has been included within the intermediate category definition for antimicrobial agents.
Intermediate

- The concept of SDD has been included within the intermediate category definition for antimicrobial agents.
- This category also includes a buffer zone, which should prevent small, uncontrolled, technical factors from causing major discrepancies in interpretations, especially for drugs with narrow pharmacotoxicity margins.
Resistant (R)

- The “resistant” category implies that isolates are not inhibited by the usually achievable concentrations of the agent with normal dosage schedules and/or that demonstrate MICs or zone diameters that fall in the range.
Nonsusceptible (NS)

- NS, is used for isolates for which only a susceptible interpretive criterion has been designated because of the absence or rare occurrence of resistant strains.
- Does not necessarily mean that the isolate has a resistance mechanism.
- For these strains, organism identification and AST results should be confirmed.
<table>
<thead>
<tr>
<th>GROUP A</th>
<th>PRIMARY TEST AND REPORT</th>
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<tbody>
<tr>
<td>Enterobacteriaceae</td>
<td>Enterococcus spp.²</td>
<td>( \text{Ampicillin}^\text{a} )</td>
<td>( \text{Ceftazidime} )</td>
<td>( \text{Azithromycin}^\text{a} ) or clarithromycin¹ or erythromycin¹b</td>
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<tr>
<td></td>
<td></td>
<td>( \text{Clindamycin}^\text{b} )</td>
<td>( \text{Oxacillin}^\text{a,b} )</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>( \text{Gentamicin}^\text{b} )</td>
<td>( \text{Cefoxitin}^\text{a,b} ) (surrogate test for oxacillin)</td>
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<tr>
<td></td>
<td></td>
<td>( \text{Tobramycin}^\text{b} )</td>
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<td>( \text{Piperacillin} )</td>
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<td></td>
<td>( \text{Trimethoprim-sulfamethoxazole} )</td>
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<thead>
<tr>
<th>GROUP B</th>
<th>OPTIONAL PRIMARY TEST REPORT SELECTIVELY</th>
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<tbody>
<tr>
<td></td>
<td>Am kacin⁶</td>
<td>Am kacin⁶</td>
<td>Ceftaroline⁷</td>
<td>Daptomycin¹²</td>
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<td>Aztreonam</td>
<td>Aztreonam</td>
<td>Linezolid</td>
<td>Linezolid</td>
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<td></td>
<td>Cefpodoxime</td>
<td>Cefpodoxime</td>
<td>Tedizolid¹³</td>
<td>Tedizolid¹³</td>
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<tr>
<td></td>
<td>Cefuroxime</td>
<td>Cefuroxime</td>
<td>Linezolid</td>
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<td></td>
<td></td>
<td>Cefuroxime</td>
<td>Doxycycline</td>
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<td></td>
<td></td>
<td></td>
<td>Minocycline¹⁰</td>
<td>Tetracycline⁹</td>
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<tr>
<td></td>
<td>Cefpodoxime</td>
<td>Cefpodoxime</td>
<td>Vancomycin</td>
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<td>Vancomycin</td>
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<td></td>
<td>( \text{Oritavancin}^\text{b} )</td>
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<td></td>
<td></td>
<td>( \text{Telavancin}^\text{b} )</td>
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<td></td>
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<td></td>
<td>Rifampin³</td>
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<tr>
<th>GROUP C</th>
<th>SUPPLEMENTAL REPORT SELECTIVELY</th>
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<tr>
<td></td>
<td>Ceftaroline</td>
<td></td>
<td>Chloramphenicol⁰</td>
<td>Gentamicin (high-level resistance testing only)</td>
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<tr>
<td></td>
<td>Chloramphenicol⁰</td>
<td></td>
<td>Ciprofloxacin or levofloxacin</td>
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<td></td>
<td>Moxifloxacin</td>
<td>Streptomycin (high-level resistance testing only)</td>
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<td></td>
<td></td>
<td>Gentamicin</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>Tetracycline⁸</td>
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<tr>
<td>Group U Supplemental for Urine Only</td>
<td>Enterobacteriaceae</td>
<td>Pseudomonas aeruginosa</td>
<td>Staphylococcus spp.</td>
<td>Enterococcus spp.&lt;sup&gt;n&lt;/sup&gt;</td>
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<tr>
<td>Cefazolin (surrogate test for uncomplicated UTI)</td>
<td>Norfloxacin</td>
<td>Norfloxacin</td>
<td>Ciprofloxacin, Levofloxacin, Norfloxacin</td>
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<tr>
<td>Fosfomycin&lt;sup&gt;e&lt;/sup&gt;</td>
<td>Norfloxacin</td>
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<td>Nitrofurantoin</td>
<td></td>
<td></td>
<td>Fosfomycin&lt;sup&gt;f&lt;/sup&gt;</td>
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<tr>
<td>Sulfisoxazole</td>
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<td></td>
<td>Nitrofurantoin</td>
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<td>Trimethoprim</td>
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<td>Sulfoxazole</td>
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<td>Nitrofurantoin</td>
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<td>Trimethoprim</td>
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<td>Tetracycline&lt;sup&gt;a&lt;/sup&gt;</td>
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Some changes in CLSI tables

- **Deleted** from Tables 1A, 1B, and/or 1C – dirithromycin, loracarbef, ofloxacin, piperacillin, quinupristindalfopristin, spectinomycin, telithromycin, and ticarcillin-clavulanate

- **Enterobacteriaceae:**
  - Added ceftriaxone-tazobactam to Test/Report Group B.
  - Clarified information for use of cefazolin as a surrogate test for uncomplicated urinary tract infections (UTIs) in Group U.
Cont’d

- **Pseudomonas aeruginosa:**
  - Moved piperacillin-tazobactam to Test/Report Group A.
  - Added ceftolozane-tazobactam to Test/Report Group B.

- **Staphylococcus spp.**:
  - Added oritavancin, tedizolid, and telavancin to Test/Report Group B.

- **Enterococcus spp.**:
  - Added oritavancin, tedizolid, and telavancin to Test/Report Group B.

- **Acinetobacter spp.**:
  - Moved tetracycline to Test/Report Group U.

- **Burkholderia cepacia complex**:
  - Moved levofloxacin and meropenem to Test/Report Group A.
  - Moved chloramphenicol to Test/Report Group C.
Cont’d

- Table 4B:
- Added QC ranges for:
  - *Haemophilus influenzae* ATCC® 49247
  - Delafloxacin
  - Lefamulin
  - Levonadifloxacin
References

- Baily and Scott 2014
- CLSI 2016
- Sciencenordic.com