The Challenge of Antimicrobial Susceptibility Testing in Iran

Mohammad Rahbar(Ph.D)

P A Professor of Clinical Microbiology

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Introduction

• Antimicrobial resistance is a critical public health and safety dilemma. We have witnessed a seemingly inexorable increase in the number of infections due to carbapenem-resistant Enterobacteriaceae, multidrug-resistant (MDR) Pseudomonas aeruginosa, MDR Acinetobacter baumannii, and vancomycin-resistant Enterococcus faecium.
Introduction

The performance of antimicrobial susceptibility testing by the clinical microbiology laboratory is important to confirm susceptibility to chosen empirical antimicrobial agents, or to detect resistance in individual bacterial isolates.
Susceptibility testing of individual isolates is important with species that may possess resistance mechanisms (eg, members of the Enterobacteriaceae, Pseudomonas species, Staphylococcus species, Enterococcus species, and Streptococcus pneumoniae. In other hand susceptibility an important issue in drug resistance surveillance.
Methods of susceptibility testing.

- **Phenotypic methods** which is widely used in clinical microbiology methods.
- **Genotypic methods**. It is only used for certain microorganisms for example defection of meca in *Staphylocococus aurues* or detection of cetaingenenes susc as coding ESBLs, KPC and ... . Molecular is difficult for standardized ant not applicable in many microbiology laboratories. This method is very expensive and needs special facilities and expertise staff.
Phenotypic methods

• *Broth dilution tests:* This method is used for detection of minimum inhibitory concentration (MIC) and is one of the earliest antimicrobial susceptibility testing methods. It was the macrobroth or tube-dilution method. This procedure involved preparing two-fold dilutions of antibiotics (e.g., 1, 2, 4, 8, and 16 µg/mL) in a liquid growth medium dispensed in test tubes.
Microdilution

- The miniaturization and mechanization of the test by use of small, disposable, plastic “microdilution” trays. It has made broth dilution testing practical and popular. Standard trays contain 96 wells, each containing a volume of 0.1 mL that allows approximately 12 antibiotics to be tested in a range of 8 two-fold dilutions in a single tray.
Fig. Microdilution
Antimicrobial gradient method

- The antimicrobial gradient diffusion method uses the principle of establishment of an antimicrobial concentration gradient in an agar medium as a means of determining susceptibility. The Etest (bioMérieux AB BIODISK)
A Staphylococcus aureus isolate tested by the Etest gradient diffusion method with vancomycin (VA), daptomycin (DM), and linezolid (LZ) on Mueller-Hinton agar.
Disk Diffusion

• The disk diffusion method is the most widely used technique in the World and is suitable for testing rapidly growing and certain fastidious bacterial pathogens.
• Integrated in the VITEK 2 system is the Advanced Expert System (AES™), a software which validates and interprets susceptibility test results, and detects antibiotic resistance mechanisms. The AES Expert System is the most developed software system in this field, and is capable of identifying even emerging and low-level resistance.
Godliness for Susceptibility testing

1-Clinical laboratory Standards Institutes (CLSI)
   Formerly CLSI 2006

2- European Committee on Antimicrobial Susceptibility Testing – EUCAST

In Our country nearly all microbiology Laboratories use CLSI guidelines.
Critical Challenges in AST

1. Pure isolate
2. Culture media: Muller-Hinton
3. Reagents: disks
4. Size of the inoculums
5. Incubation condition
6. Control with reference strains
7. Reading inhibition diameters (accurate measurement)
8. Knowledge of staff
Patient results may be incorrect if

- The organism was misidentified
- Misidentification leads to chose wrong antibiotic disks for susceptibility testing
- If mix microorganisms was tested
- Mixed organism testing results different zone of inhibition or growth inside of zone of inhibition.
Mueller-Hinton Agar

• It shows acceptable batch-to-batch reproducibility for susceptibility testing.
• It is low in sulfonamide, trimethoprim, and tetracycline inhibitors.
• It gives satisfactory growth of most nonfastidious pathogens.
MHA

Unfortunately some of MHA culture media in our country have not good quality and all user should provide it from approved companies and sources.

performance of quality control MHA is also mandatory
Antibiotic disks

- Stored and handled correctly
  - Refrigeration – taken out 1 hour before use
- Expiry dates noted
- Disks at room temperature before use
  - Avoid condensation
- Placing of disks within **15** minutes of swabbing
Reading Plates and Interpreting Results

- If cefoxitin (35°C) is being tested against Staphylococcus spp. or vancomycin against Enterococcus spp., **24 hours of incubation** are required before reporting as susceptible; other agents can be read and reported at **16 to 18 hours**.
A question

Which companies produce good quality Antibiotic disks
Wide variation in disk quality in 16 selected disks from nine manufacturers.

EUCAST Development Laboratory (EDL)
Växjö
Sweden
23 October 2015
Notice

The aim of our for showing these results is not confirmation or rejection of companies, for this reason the name of manufactures has been deleted and shown as 1, 2, …………….9
Disk diffusion testing

- Nine manufacturers were asked to supply disks for the 16 selected antimicrobial agents and nominal disk potencies. Not all manufacturers supplied all disks. Disk diffusion testing was performed by the EDL in April – September 2014. The results from this study was presented as a poster at ECCMID 2015 (P1239), with manufacturers anonymized.
Disk diffusion was performed according to EUCAST methodology.

Each disk was tested against all QC strains with targets and ranges to the agent in the EUCAST Quality Control Tables v. 5.0.

For each agent, all disks supplied for the study were placed on a single 150mm circular agar plate, except for meropenem 10 µg, for which two plates were used due to large inhibition zones.

Each disk-strain combination was tested in triplicate (three individually prepared inoculum suspensions) on the same day.

All tests were performed on in-house prepared Mueller-Hinton agar plates (MH and MH-F as recommended) using MH agar from two manufacturers.
# Antimicrobial disks and QC strains

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<th>Antimicrobial agent</th>
<th>Disk content (µg)</th>
<th>QC Strain</th>
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7/25/2017
Manufacturers and disk lots First study, 2014

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**NA = Not Available**
## Results first study, 2014

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</table>

### Notes:

- **Mean value within ± 1 mm of the target value**
- **Mean value > 1 mm but within ± 2 mm of the target value**
- **Mean value > 2 mm from target value but still within the QC range**
- **Mean value out of the QC range**

¹ EUCAST has evaluated and, if acceptable, adopted the CLSI quality control ranges for disk diffusion tests. In cases where EUCAST recommendations for strains, medium or disk contents differ from those of CLSI, EUCAST has developed separate criteria.

² Since these results were presented at ECCMID 2015, the QC range for *H. influenzae* ATCC 49766 and cefotaxime 5 µg has been revised.

7/25/2017
Conclusion

• The results from this study show that there are significant differences in antimicrobial activity in disks from different manufacture.

• Disk manufacturers must continuously review their disk manufacturing process.

• User should perform quality control disk roundly as recommended by CLSI or EUCAST
Reference Strains for QC of antibiotic disks

- *E. coli* ATCC 25922
- *S. aureus* ATCC 25923
- *P. aeruginosa* ATCC 27853

QC organisms must be obtained from reputable source
The laboratory must test and report the antimicrobial agents that are most appropriate for the isolated organism and site of the infection, and the institution’s formulary.
SELECTION OF DRUGS FOR ROUTINE TESTING

- The CLSI provides tables that list the agents appropriate for testing members of the Enterobacteriaceae, Pseudomonas, and other gram-negative, nonfermenters, staphylococci, enterococci, streptococci, Haemophilus species, etc. The listings include recommendations for agents that are important to test routinely, and those that may be tested or reported selectively based on the institution’s formulary.
SELECTION OF DRUGS FOR ROUTINE TESTING

• The availability of antimicrobial agents for testing by the laboratory’s routine testing methodology must next be determined. The disk diffusion and gradient diffusion procedures offer the greatest flexibility including testing of newly available drugs. In our country, disk of some antibiotics are not available for testing. (Linozolid, daptomycin, azteronam .......)
Reading Plates and Interpreting Results

- Zones are measured to the nearest whole millimeter, using sliding calipers or a ruler, which is held on the back of the inverted petri plate. The petri plate is held a few inches above a black, nonreflecting background and illuminated with reflected light.
Factors Affecting Size of Zone of Inhibition

- Potency of antibiotic discs
- Composition of medium
- Acidic pH of medium
- Alkaline pH of medium
- Reading of zones

- Deterioration in contents leads to reduced size
- Affects rate of growth, diffusion of antibiotics and activity of antibiotics
- Tetracycline, novobiocin, methicillin zones are larger
- Aminoglycosides, erythromycin zones are larger
- Subjective errors in determining the clear edge
MEASURING CONDITIONS

Calipers

- read with good light, and from the back of the plate
- zone size reading is drug specific
- magnification may help
- millimeters matter

Ruler
The results of a susceptibility test must be interpreted by the laboratory prior to communicating a report to a patient’s physician. Optimal interpretation of MICs requires knowledge of the pharmacokinetics of the drug in humans, and information on the likely success of a particular drug in eradicating bacteria at various body sites.
This is best accomplished by referring to an expert source such as the CLSI or EUCAST which publishes interpretive criteria for MICs of all relevant antibiotics for most bacterial genera. Indeed, both MIC values and disk diffusion zone diameters must be interpreted using a table of values that relate to proven clinical efficacy of each antibiotic and for various bacterial species.
The CLSI zone size and MIC interpretive criteria are established by analysis of 3 kinds of data: (1.) microbiologic data, including a comparison of MICs and zone sizes on a large number of bacterial strains, including those with known mechanisms of resistance that have been defined either phenotypically or genotypically; (2) pharmacokinetic and pharmacodynamic data; and (3) clinical studies.
COMMON INTERPRETATION PROBLEMS

Results depend on the technique used.

Many factors influence results:

• Lack of standardization of the inoculums
• Thickness and quality of the culture media
• Quality and conservation of the disks
• Quality control with standardized strains
• Condition and duration of incubation
NEED FOR MODIFIED METHODS

- Modified Methods in Disk diffusion for Antibiotic sensitivity testing to be used for detections of following bacterial isolates
- 1 MRSA
- 2 ESBL
- 3 Enterobacteriaceae and Gram negative bacteria and Carbapenems resistant using Modified Hodge test
WHAT IS THE ACCEPTABLE ACCURACY OF A SUSCEPTIBILITY TEST METHOD

• It is important that the tables used for susceptibility test interpretations represent the most current criteria. Indeed, the CLSI documents are reviewed and updated frequently, usually once per year. Use of old or outdated information from the original editions of FDA-approved drug labels or older CLSI tables could represent a serious shortcoming in the reporting of patients’ results.
WHAT IS THE ACCEPTABLE ACCURACY OF A SUSCEPTIBILITY TEST METHOD

• When assessing the accuracy of various susceptibility testing methods as compared to standard reference methods, the terms very major and major errors have been used to describe false susceptible or false-resistant results, respectively.
WHAT IS THE ACCEPTABLE ACCURACY OF A SUSCEPTIBILITY TEST METHOD

• In evaluations of new susceptibility testing methods it is important to examine a representative number of strains that are resistant to various drugs to verify the ability of the new test to detect resistance and to test a number of susceptible strains to determine the rate of major errors that might be expected in a typical clinical laboratory setting
Lack of break point for some microorganisms

There is not a defined break point for some antibiotics

There is not method of susceptibility testing for some microorganism (Bacillus spp,....)
WHAT IS THE ROLE OF MICROBIOLOGY DEPARTMENTS

• Each laboratory should have a **staff member** with the time, interest, and expertise to provide leadership in antibiotic testing and resistance. This person would read relevant publications, network with other laboratories, and evaluate potentially useful tests to detect new forms of resistance before **new CLSI-recommended tests become available**
