AST Antimicrobial Susceptibility Testing

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The purpose

- To present the methods used for AST
  - B - Agar dilution
  - C - semi-automated methods
  - D - Agar diffusion
  - E - Gradient diffusion

- To define for each one
  - Parameters
  - Performances
  - Advantages
  - Disadvantages

- Only rapid growing bacteria
Choice of routine methods

- For non fastidious bacteria all methods are satisfactory
- Depends on:
  - cost
  - Time of processing/work
  - Reagents availability
  - Professional knowledge
  - Automation access
- More sophisticated methods are:
  - Micro-dilution
  - Agar dilution
  - Semi-automated
- Disk diffusion remains the more accessible and economic method
- Most laboratories have 2 methods

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MIC = Minimum Inhibitory Concentration

Standard inoculum
Doubling dilutions of antibiotic (mg/L)

64  32  16  8  4  2  1  .5

4 μg/ml

Adapted From Pr C. Block

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Broth Micro-dilution - 1

- Broth Muller-Hinton micro-dilution method is the international standard reference method (ISO 20776-2006)
- This standard was chosen by the
  - ISO
  - EUCAST
  - CLSI
  - CA-SFM
- The method was very carefully evaluated and
- Was correlated to modern parameters like pharmokinetics/pharmacodynamics (PK/PD) (breakpoints)

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Broth Microdilution-2

- Microplates 96 wells U
- In house or reagents
- In house only Reference Lab (titrated antibiotic powder, QC)
- ISO 20776-1(2006) 96 euros
- 100 µl MH broth cation-adjusted (CAMHB), 5x10^4 bacteria/ml, 35°C 18-24h.
- QC +++ ATCC and QC target /range ± 1 dilution
An example of broth micro-dilution

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Broth Micro-dilution-3

- **Other media**
  - several genus needs enriched broth
    - HTM for *Haemophilus* spp
    - Brucella for *Brucella* and anaerobies
    - Lysed horse blood
    - Special cases: Daptomycin, *Abiotrophia*, *Granulicatella* (B6)...
  - Quite simple to adapt the method for each purpose!

- **Problems**
  - Not “clear cut” (“En traine”)
  - Partial growth (80% control)
  - Well(s) with no growth

- **MBC (minimal bactericidal concentration)?**
  - Important to characterize new antibiotics
  - Bacteriostatic or bactericidal
  - Not useful to determine in clinical practice

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Agar dilution method-1

- Standard method published: CLSI, EUCAST, CA-SFM

42 tips in each

Antibiotic included in the agar plate
Each plate represents another dilution

Steers apparatus

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Agar dilution method

- Very frequently used method
- Mueller Hinton plates, Isosensitest etc...
- No international standardization

Problems:
Not standardized for: *H. influenzae* and *parainfluenzae*, *B. cepacia* and other fastidious bacteria
- For some bacteria/antibiotic diff. between MIC
- Technical problems
- Ionic concentration not controlled, PB with Dapto. and Tigecycline.

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Semi-automated methods-1

- 3 Apparatus: Siemens MicroScan®
  Biomerieux Vitek2®, BD Phoenix®
- Liquid medium growth and turbidimetric reading
- Antibiotic concentrations are chosen between breakpoints
- Validation versus the micro-dilution method
- Correlation with the Ref. method is software assisted

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Semi-automated methods-2

- Microscan® and BD Phoenix® → true MIC, but only 1 or 2 dilutions around breakpoints
- Biomerieux Vitek2® → growth algorithm
- Whatever, limitation in the results
- Limitation of the card panel
- Delay between occurrence of new events and updates.
- Cost is higher than other phenotypic methods.
The most used routine method for routine AST
Available for a lot of bacteria species, fastidious bacteria included (Streptococcus, Haemophilus, pneumococcus...)
A lot of International experience and experts from all over the world.
A lot of variation on the same principle. But now, EUCAST and CLSI
Agar diffusion method-2

Regression curve

Zone diameter (mm)

Adapted From Pr C. Block

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Regression depends on the species but the method uses an average RC.

There is a need to use RC according to the species (EUCAST)

Some antibiotics exhibit diffusion problems (glycopeptides, polypeptides, daptomycin, linezolide) so Disc Diffusion is not adapted.

The USA standard is CLSI ($375!!) widespread

European standard is EUCAST (Free of charge), rather new, but progressively replaces national European standards (BASC, CA-SFM, SRGA)
Agar diffusion method-4

- CLSI (Clinical and Laboratory Standard Institute)
  - Mueller Hinton → rapid growing bacteria
  - *Haemophilus* Test Medium.
  - MH + 5% sheep blood → *Streptococcus (pneumoniae)*
  - Other fastidious bacteria

- EUCAST (European Union Committee for AST)
  - Mueller Hinton → rapid growing bacteria
  - MH + 5% horse blood + 20mg β-NAD/L → *Haemophilus AND Streptococcus (pneumoniae)*
  - Correlation between Zone Diameters and MIC
  - Breakpoints from EUCAST
Agar diffusion method – 5

- Standardization +++
- Standard Operating Procedures
- Zone Diameter Distribution
- CLSI and EUCAST: same MIC/ZD for rapid growing bacteria
- CLSI and EUCAST: same MIC for fastidious but not same ZD.
- For better validation, comparison with histogram distribution
- QC for routine is necessary to implement.

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Agar diffusion method - Performances

**FIGURE 2.4** Relationship between zone diameters and MICs.

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Agar diffusion method

Antimicrobial wild type distributions of microorganisms

Search

Method:  
- MIC
- Disk diffusion

Antimicrobial:  
Species:  

Species: Staphylococcus aureus (Method: Disk diffusion)

Distributions include collated data from multiple sources, geographical areas and time periods and can never be used to infer rates of resistance

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**Imipenem / Klebsiella pneumoniae**

EUCAST zone diameter distribution - Reference database 2012-10-28

EUCAST disk diffusion method

Distributions include collated data from multiple sources, geographical areas and time periods and can never be used to infer rates of resistance

---

**Zone diameter (mm)**

% microorganisms

**ECOFF**

Disk content: 10

Epidemiological cut-off: WT ≥ 23 mm (MIC ≤ 1 mg/L)

Clinical breakpoints: S ≥ 22 mm, R < 16 mm (S ≤ 2 mg/L, R > 8 mg/L)

441 observations (6 data sources)

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Interpretative reading of the antibiogram

**BETA-LACTAMINES et ENTEROBACTERIES**

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**IBL : Inhibiteur de Bêta-Lactamase**

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This is a sub-population

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E- Gradient Diffusion

- 1988, new method antibiotic gradient strip E-test® (AB-Biodisk)
- Also fungus and fastidious etc..
- Apparently, simple to use but needs to be performed according to manufacturer instructions and to implement QC/reading
- Quite expensive but simply allows to obtain a very good approximation of MIC for a lot of microorganisms

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Conclusions

- Micro-dilution and Agar dilution → true MIC but not possible to use in routine

- For routine
  - Semi-automated method → good approximation of the MIC but expensive, not flexible. OK for SOP. Problems for detection of new mechanisms of resistance.
  - Disk diffusion → not MIC but EUCAST approach is of interest, low cost but needs carefully implementation of SOP and QC. Need for another method like E-test for some special cases (glycopeptides, polypeptides, etc...).
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