MIC-determination

- Definitions: MIC- and diffusion methods
- Standardisation
- Clinical breakpoints and epidemiological cut-off values
- MIC contra disk diffusion
- Sensititre

Definitions

MIC methods

MIC: Minimal Inhibitory Concentration

The lowest concentration that is able to inhibit growth of the bacteria

Two fold dilutions of antimicrobial → inoculate + incubate →
the lowest conc. with no visible growth is read as the MIC

- microdilution MIC (microtitre wells, Sensititre)
- agar dilution (also called plate dilution)
- macrodilution MIC (in tubes)

Diffusion methods

Agar plate is inoculated and antimicrobial diffuses from a disk or from a paperstrip in to the agar. Next day the growth inhibitionzone is read.

- disk diffusion
- E-test

Standardisation

All methods are extremely sensitive to variations in performance.
Many factors influence the result:

- Size of inoculum
- Contents and acidity (pH) of the broth or agar
- Incubation time and temperature
- Reading procedures

And further more for the diffusion methods:

- Growth rate of the bacteria
- Diffusion rate of the antimicrobial into the agar
- Depth of the agar
- Age of the agar

Reliable and reproducible data = Standardized methods and daily QC !

The CLSI standards

- International standard that describes the methods in details
  - media, inoculum, incubation, etc. - both disk diffusion and MIC
  - forexample acidity 7.2-7.4 and agar depth 4 mm…

- CLSI has defined QC referencestrains and the acceptable QC results for these strains.

- CLSI recommends clinical breakpoints for interpretation of the result

Resistant: Treatment failure can be expected.

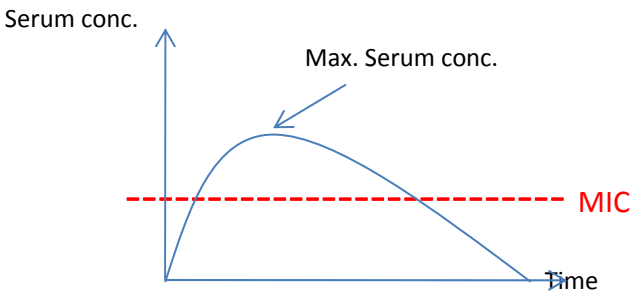
Sensitive: Succesfully treatment can be expected.

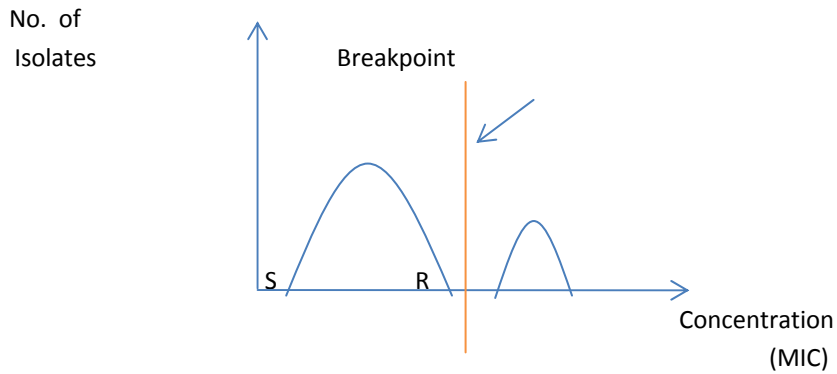
Intermediary: Treatment is possible if the infection is in bodysites where the antimicrobial is concentrated.
Primarily a bufferzone to avoid misinterpretation

Clinical breakpoints

- Are established according to

- the serum conc. obtained with normal dosage





- and the MIC distribution for the bacteria

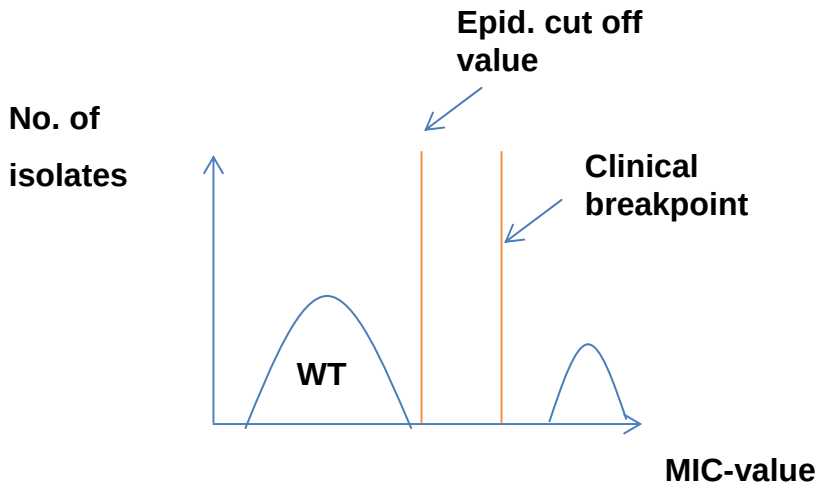
- CLSI has not applied breakpoints for all antimicrobials and for all bacteria

Epidemiological cut off values

European counterpart to CLSI: www.EUCAST.org

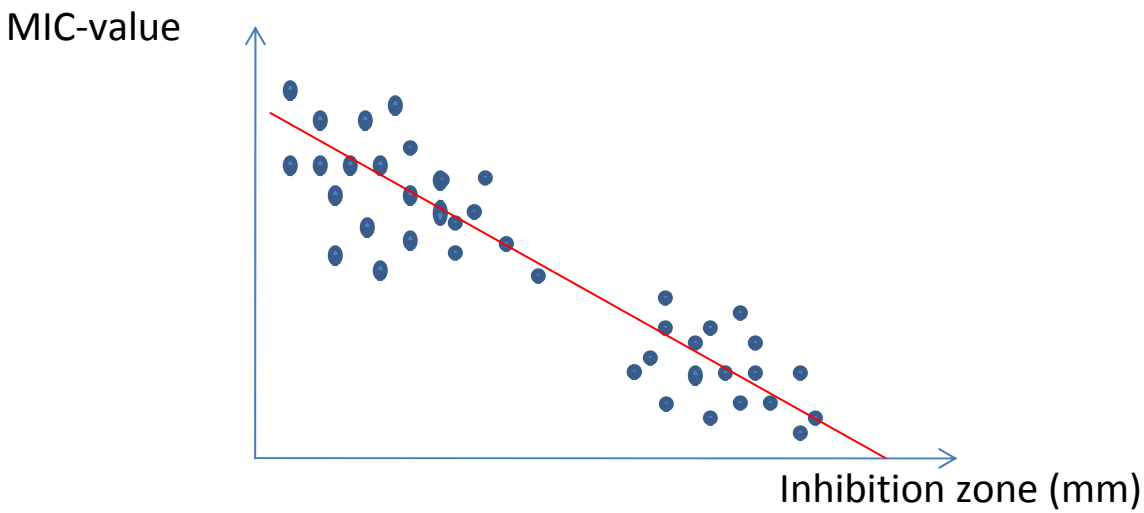
- Uses CLSI methods, QC referencestrains etc
- Defines species-specific clinical breakpoints
- Defines epidemiological cut off values
  - for monitoring purpose only, no relation to clinical data
  - defined by the WildType distribution of the bacteria
  - all isolates with MICs above the WT distribution are called resistant !

Illustration of epid. cut off values



Diffusion tests are fine…

- If standardized methods are used and QC performed
- The methods are correlated to MIC by regressionlines for each antimicrobial for both slow growing and fast growing bacteria.



Ampicillin

Fast growing bacteria

MIC contra agar diffusion

MIC determination

- Golden standard for AST
- Data more reproducible
- Better separation of R/S
- More information
- Expensive
- Only pure cultures
- Contaminations more difficult to detect

Diffusion methods

- Cheaper
- Primary material
- See contaminations
- Quick screening (4 hours)
- Qualitative information: ”Resistant or not”
- Less reproducible data
- Standardisation more difficult

Sensititre-system

- Kommercially available microdilution MIC in microtitrewells,
  Trek Diagnostics, UK

- Two fold dilution of dehydrated antimicrobials – just add the bacteria susp.

- The Sensititre-system is standardized according to the CLSI standard
  and the system is validated by the FDA in the USA

- The system consists of:
  - MIC panels (custom designed or pre-designed by Trek)
  - nephelometer and MacFarland 0.5 standard
  - autoinoculator
  - tubes, dosing heads and broth
  - Sensitouch and software for reading the panels
  - Service visit by Trek Diagnostics once a year