Methods for susceptibility testing

I. Phenotypic test methods

- **MIC determination (broth micro dilution, gradient tests, disk diffusion, automated and semiautomated systems such as Vitek2, Phoenix, Microscan)**
- based on **antimicrobial activity (MIC)** and **breakpoints**

- Predict susceptibility and resistance.
- Quantifiable.
- Require standardisation.
- Require breakpoints and breakpoints require agreement.

Kahlmeter-G, 2014 ASA Meeting

Phenotypic susceptibility testing is based on

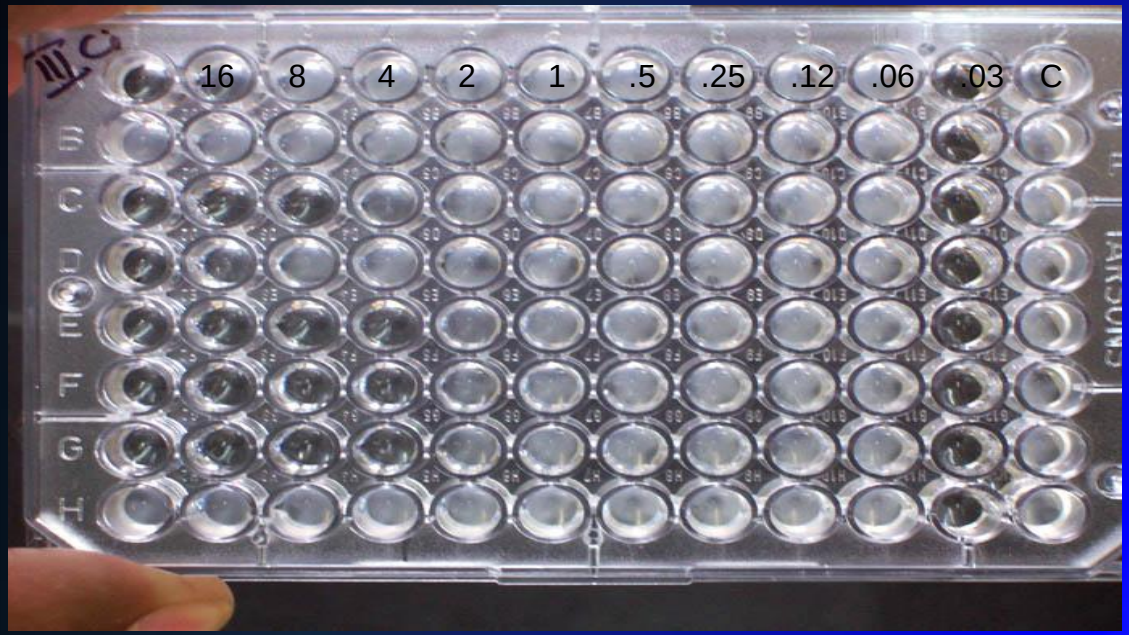
MIC + breakpoints

↓ ↓

Standardisation — ISO standard

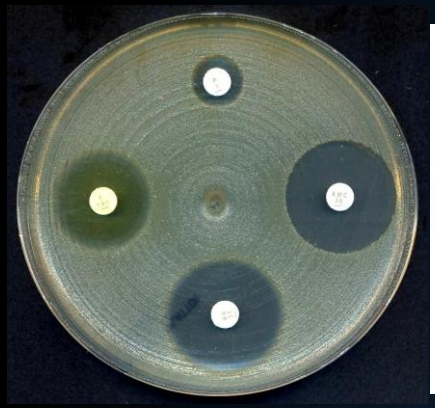
Agreement — Breakpoint committees

MIC determination
Broth microdilution in accordance with the
ISO-standard*



See the ISO, CLSI or EUCAST websites

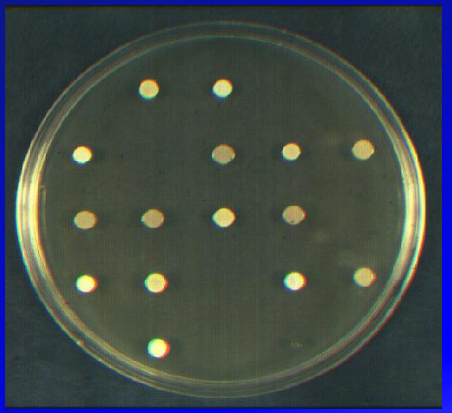
All other tests are surrogate MIC determination



Disk diffusion’

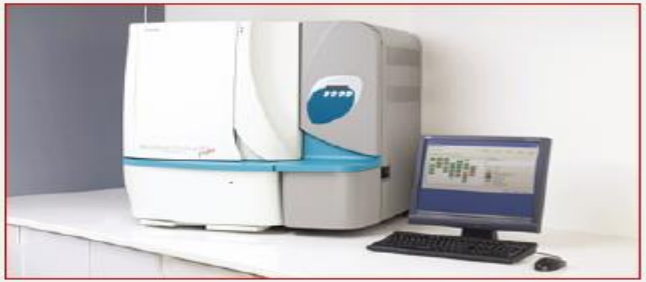
Gradient MIC test:

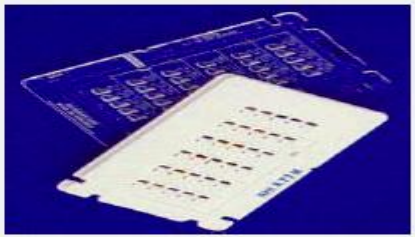
Several manufacturers:
bioMerieux (Etest)
Oxoid (M.I.C.E.)
Liofilchem (MIC-strip)



Agar dilution

All automated AST machines are surrogate methods





Clinical breakpoints and ECOFFs

Clinical breakpoints vs. ECOFFs

ECOFF

The ECOFF is the highest MIC value of isolates devoid of phenotypically expressed resistance.

- Wild type ≤X mg/L (X=ECOFF)
- Non wild type >X mg/L

Clinical breakpoints

An MIC concentration defined by man to predict clinical success and failure

- S ≤Y mg/L
- R >Y mg/L

Tools for determining clinical breakpoints

- Dose and mode of administration
- Clinical targets (indications)
- Target organisms (indications)
- MIC distributions of target organisms
- Resistance mechanisms of clinical importance in target organisms
- Pharmacokinetics of agent in target patients
- Pharmacodynamics of agent in relation to target organism
- Clinical outcome data for target infections

S, I and R

- **Susceptible (S)**
  - a micro-organism is defined as susceptible by a level of antimicrobial activity associated with a high likelihood of therapeutic success
- **Intermediate (I)**
  - a micro-organism is defined as intermediate by a level of antimicrobial agent activity associated with uncertain therapeutic effect.
    It implies that an infection due to the isolate may be appropriately treated in body sites where the drugs are physically concentrated or when a high dosage of drug can be used; ~~(it also indicates a buffer zone that should prevent small, uncontrolled, technical factors from causing major discrepancies in interpretations.)~~
- **Resistant**
  - a micro-organism is defined as resistant by a level of antimicrobial activity associated with a high likelihood of therapeutic failure.

Breakpoints are determined by:

1. Medicines agencies (EMA, FDA)
2. Breakpoint committees

Pharmaceutical companies

AST companies

Colleagues who know better

EUCAST
EUROPEAN COMMITTEE ON ANTIMICROBIAL SUSCEPTIBILITY TESTING
European Society of Clinical Microbiology and Infectious Diseases

ESCMID
EUROPEAN SOCIETY OF CLINICAL MICROBIOLOGY AND INFECTIOUS DISEASES

EUCAST General Committee
All European Countries + Australia + USA + …

EUCAST Steering Committee
National Breakpoint Committees and GC members

ecdc
EUROPEAN CENTRE FOR DISEASE PREVENTION AND CONTROL

EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

Expert groups
- M.tuberculosis
- Helicobacter
- C.difficile
- etc

Subcommittees

Antifungal susceptibility testing
Anaerobes
Expert Rules and Intrinsic Resistance
Detection of resistance mechanisms
The relationship between phenotype and genotype
Veterinary breakpoints (VetCAST)

NAC

- **Antimicrobial susceptibility testing**
  - **Coherent strategy** at national level
  - **Implementation** of breakpoints and methods
  - **Education** (national workshops, websites)
  - **Translation** of documents
  - **Liaison** and **consultation** with EUCAST – via the General Committee and open consultations
  - Liaison with other national groups involved in antimicrobial stewardship or surveillance of resistance.
  - QA

- (Antimicrobial Policies)
- (Antimicrobial Resistance Surveillance)
- (Antimicrobial Consumption and Stewardship)

Antibiotics without breakpoints

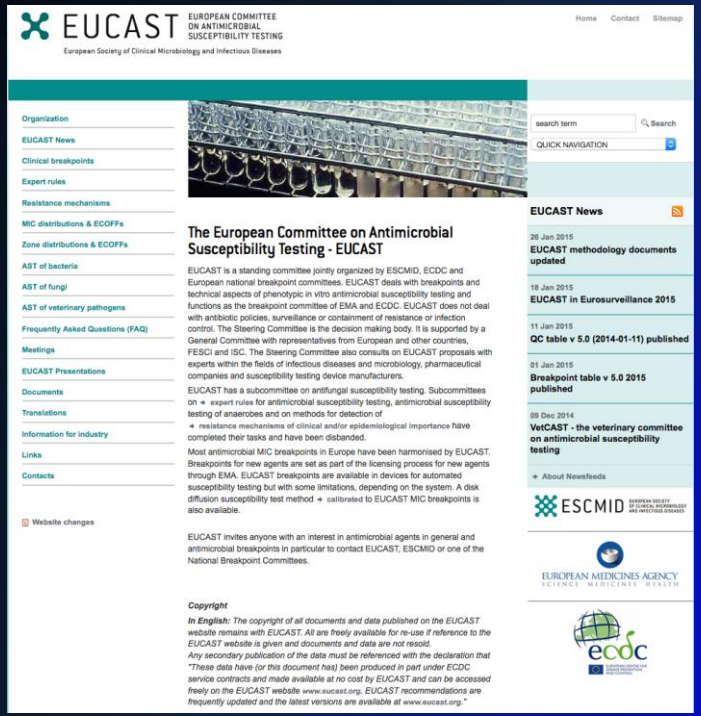
- Spiramycin
- Josamycin
- Cefoperazone-sulbactam
- ….

- Currently EUCAST is preparing a list of “all” globally available antibiotics with the rationale for why they were not given breakpoints

Bacteria without breakpoints

- Aerococcus spp - 2015
- Kingella kingae - 2015
- M.tuberculosis - ongoing
- Actinomyces spp
- Nocardia spp
- ….

EUCAST Websites



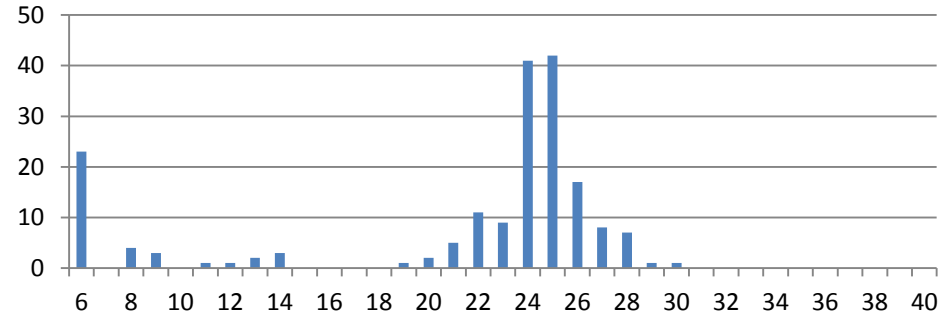
EUCAST websites are

- www.eucast.org
- Free of charge
- No login
- Updated weekly
- Newsflow (RSS)
- MIC wild type distributions
- >50 000 visits/month

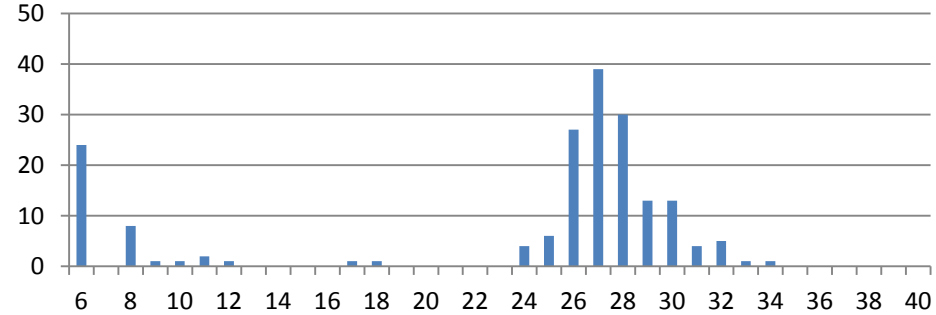
Analysis of results from rapid AST

E. coli with cefotaxime 5 µg

6 h

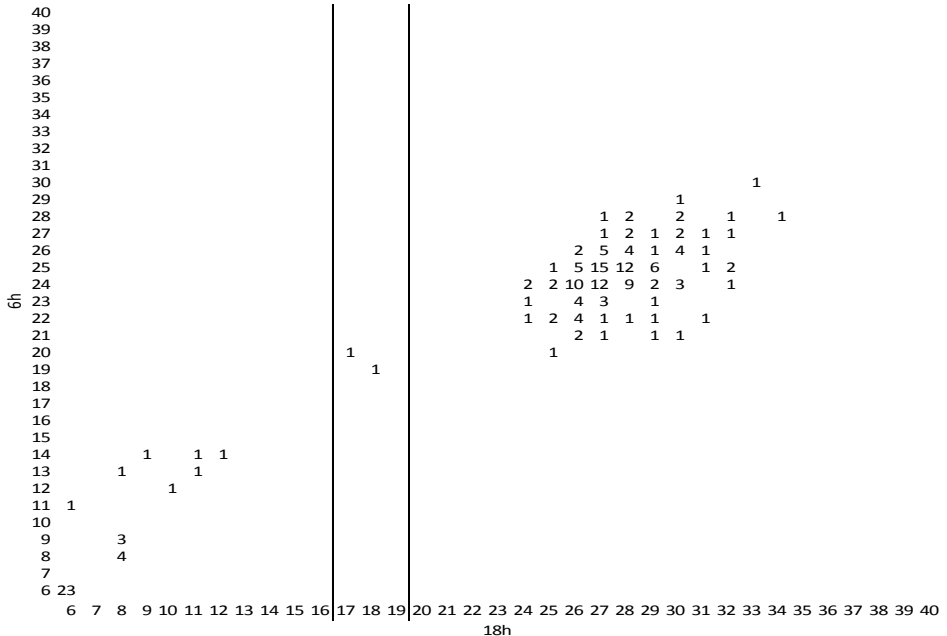


18 h



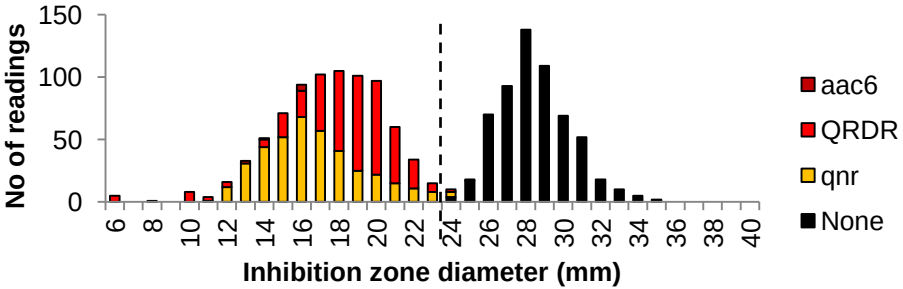
On-going work:

- Comparison of medians (6 h vs. 18 h)
- Correlation with standardised DD
- Selection of isolates with low-level resistance for further testing

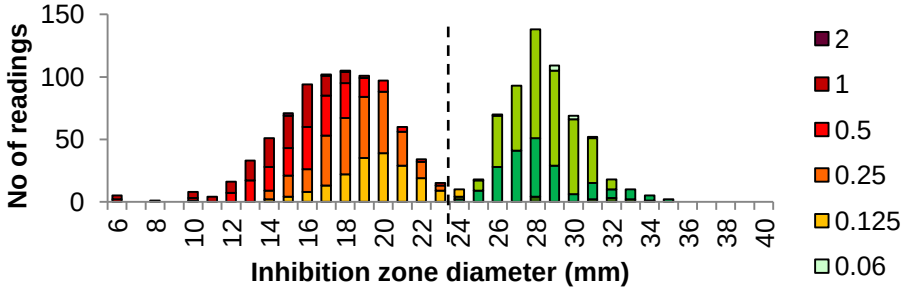


Salmonella spp.: Pefloxacin and ciprofloxacin disk diffusion testing

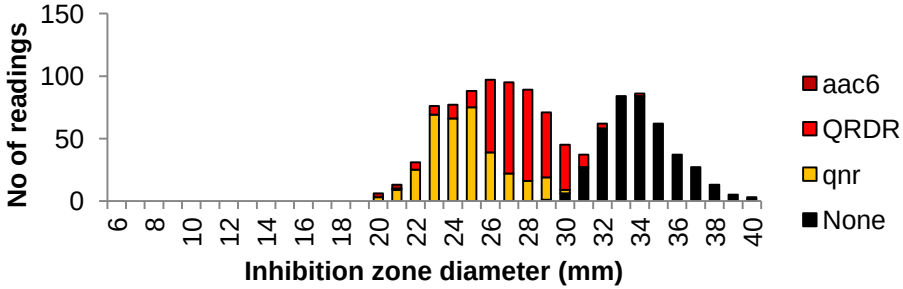
Pefloxacin 5 µg vs. FQ resistance mechanism
***Salmonella* spp., 153 isolates (1391 readings)**



Pefloxacin 5 µg vs. Ciprofloxacin MIC
***Salmonella* spp., 153 isolates (1391 readings)**



Ciprofloxacin 5 µg vs. FQ resistance mechanism
***Salmonella* spp., 153 isolates (1104 readings)**



Ciprofloxacin 5 µg vs. Ciprofloxacin MIC
Salmonella spp., 153 isolates (1104 readings)