

# 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.

# Phenotypic susceptibility testing is based on

MIC

+

breakpoints



Standardisation

ISO standard

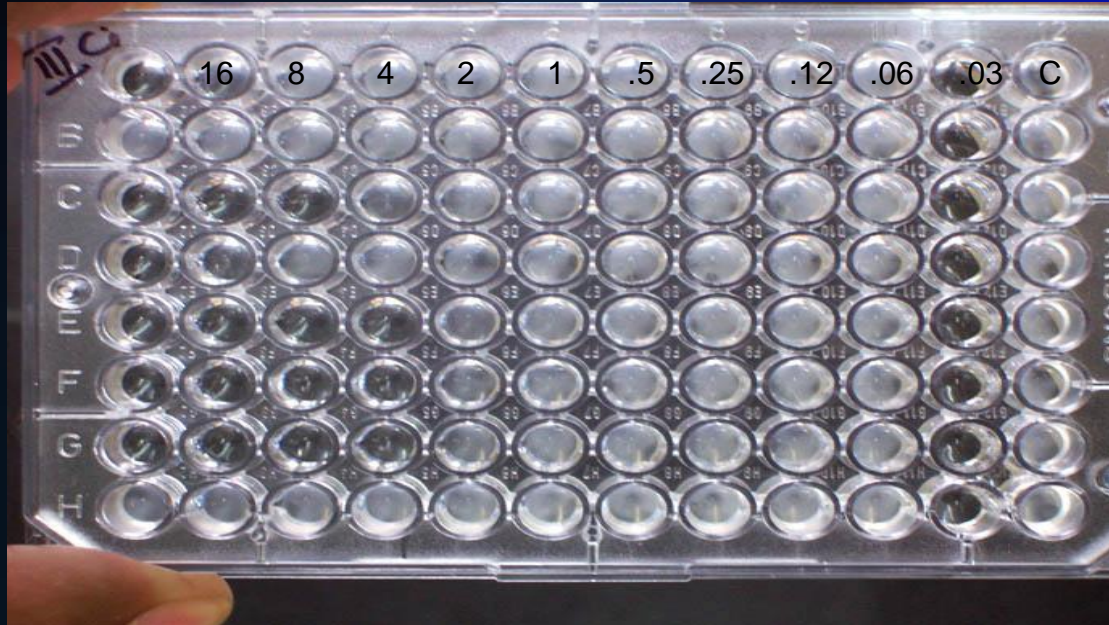


Agreement

Breakpoint committees

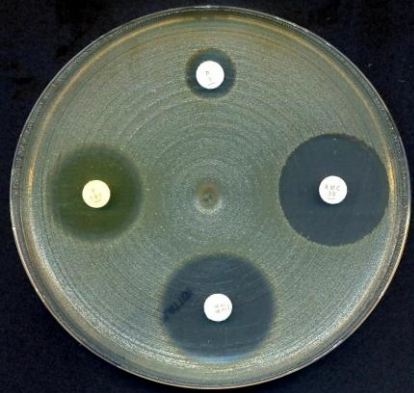
# MIC determination

## Broth microdilution in accordance with the ISO-standard\*



See the ISO, CLSI or EUCAST websites

# All other tests are surrogate MIC determination

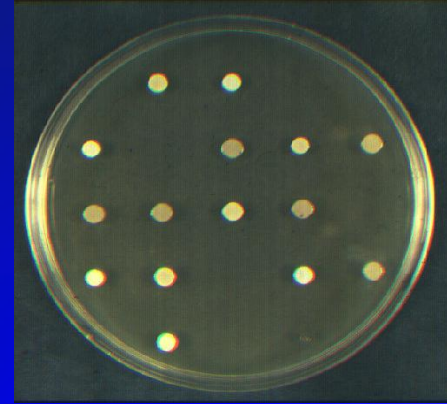


**Disk diffusion'**



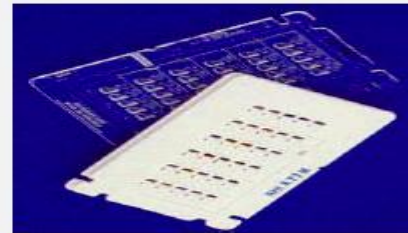
**Gradient MIC test:**

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  $\leq X$  mg/L ( $X = \text{ECOFF}$ )
- Non wild type  $> X$  mg/L

## Clinical breakpoints

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

- S  $\leq Y$  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



### Subcommittees

Antifungal susceptibility testing

Anaerobes

Expert Rules and Intrinsic Resistance

Detection of resistance mechanisms

The relationship between phenotype and genotype

Veterinary breakpoints (VetCAST)

### Expert groups

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

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

Home Contact Sitemap

Organization  
EUCAST News  
Clinical breakpoints  
Expert rules  
Resistance mechanisms  
MIC distributions & ECOFFs  
Zone distributions & ECOFFs  
AST of bacteria  
AST of fungi  
AST of veterinary pathogens  
Frequently Asked Questions (FAQ)  
Meetings  
EUCAST Presentations  
Documents  
Translations  
Information for industry  
Links  
Contacts

Website changes

**The European Committee on Antimicrobial Susceptibility Testing - EUCAST**

EUCAST is a standing committee jointly organized by ESCMID, ECDC and European national breakpoint committees. EUCAST deals with breakpoints and technical aspects of phenotypic in vitro antimicrobial susceptibility testing and functions as the breakpoint committee of EMA and ECDC. EUCAST does not deal with antibiotic policies, surveillance or containment of resistance or infection control. The Steering Committee is the decision making body. It is supported by a General Committee with representatives from European and other countries, FESCI and ISG. The Steering Committee also consults on EUCAST proposals with experts within the fields of infectious diseases and microbiology, pharmaceutical companies and susceptibility testing device manufacturers.

EUCAST has a subcommittee on antifungal susceptibility testing. Subcommittees on = expert rules for antimicrobial susceptibility testing, antimicrobial susceptibility testing of anaerobes and on methods for detection of = resistance mechanisms of clinical and/or epidemiological importance have completed their tasks and have been disbanded.

Most antimicrobial MIC breakpoints in Europe have been harmonised by EUCAST. Breakpoints for new agents are set as part of the licensing process for new agents through EMA. EUCAST breakpoints are available in devices for automated susceptibility testing but with some limitations, depending on the system. A disk diffusion susceptibility test method = calibrated to EUCAST MIC breakpoints is also available.

EUCAST invites anyone with an interest in antimicrobial agents in general and antimicrobial breakpoints in particular to contact EUCAST, ESCMID or one of the National Breakpoint Committees.

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26 Jan 2015  
**EUCAST methodology documents updated**

18 Jan 2015  
**EUCAST in Eurosurveillance 2015**

11 Jan 2015  
**QC table v 5.0 (2014-01-11) published**

01 Jan 2015  
**Breakpoint table v 5.0 2015 published**

09 Dec 2014  
**VeCAST - the veterinary committee on antimicrobial susceptibility testing**

About Newsfeeds

ESCMID EUROPEAN SOCIETY OF CLINICAL MICROBIOLOGY AND INFECTIOUS DISEASES

EUROPEAN MEDICINES AGENCY  
SCIENCE MEDICINES HEALTH

ecdc EUROPEAN CENTRE FOR DISEASE PREVENTION AND CONTROL

## EUCAST websites are

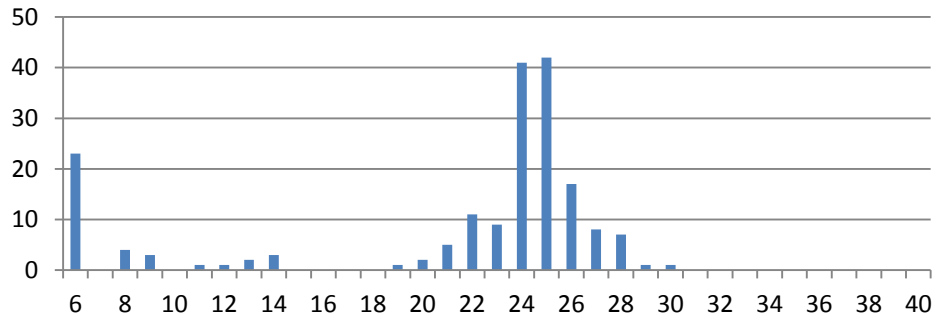
- [www.eucast.org](http://www.eucast.org)
- Free of charge
- No login
- Updated weekly
- Newsflow (RSS)
- MIC wild type distributions
- >50 000 visits/month



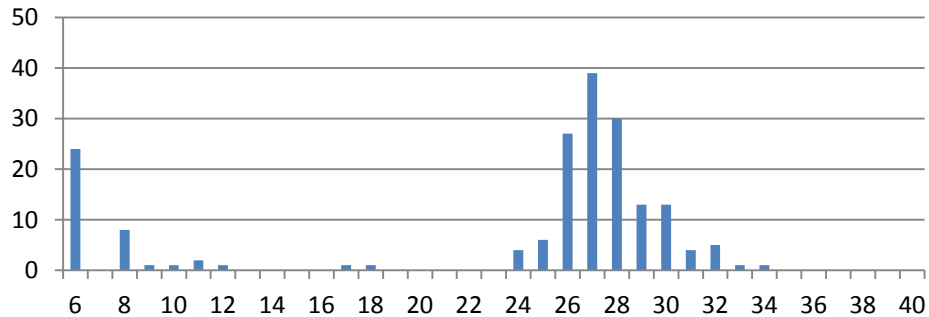
# Analysis of results from rapid AST

*E. coli* with cefotaxime 5  $\mu$ g

6 h

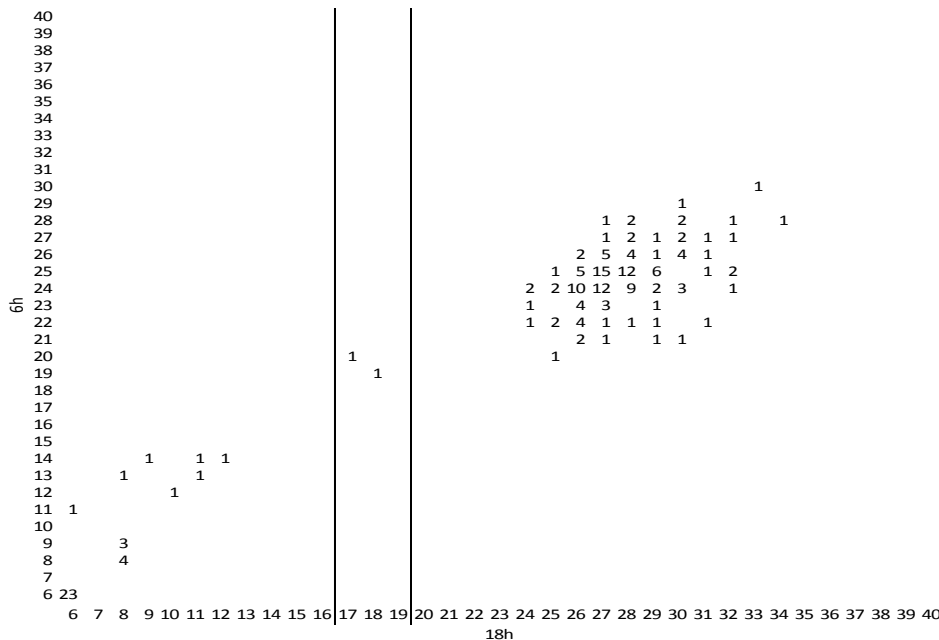


18 h



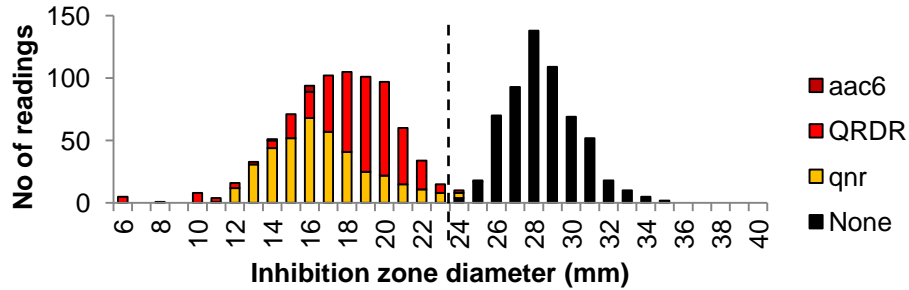
## On-going work:

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

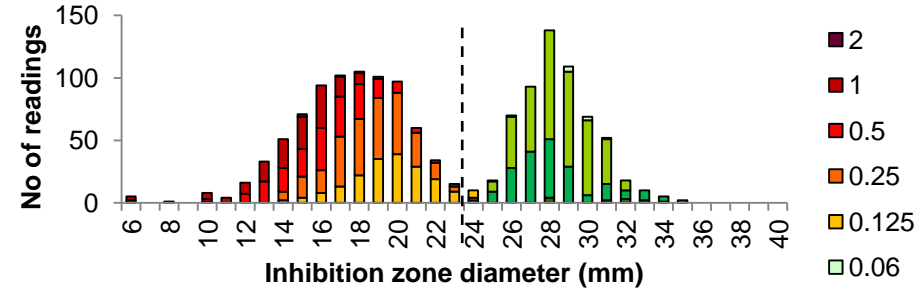


# Salmonella spp.: Pefloxacin and ciprofloxacin disk diffusion testing

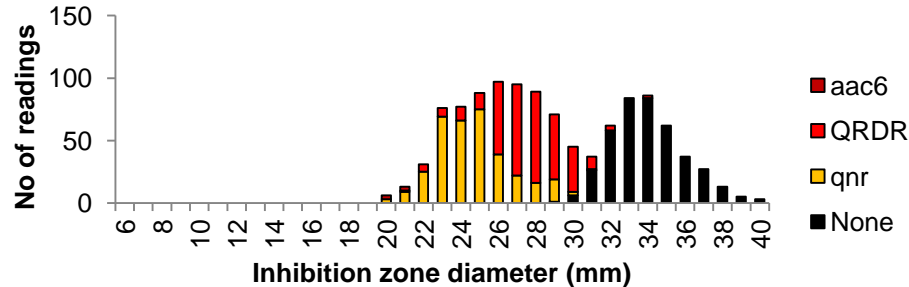
## Pefloxacin 5 µg vs. FQ resistance mechanism *Salmonella* spp., 153 isolates (1391 readings)



## 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)

