What to expect of the ”future” in antimicrobial susceptibility testing?

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The Future

- Any new techniques in the foreseeable **future**?
- Any promising ”**rapid**” methods around?
- Global harmonisation of **breakpoints**?
- Global harmonisation of **ECOFF**s?
- Global harmonisation of **methods**?
- Any promising true **automation**?
- Is **TATFAR** (transatlantic taskforce on AR) signalling progress?
- **EUCAST** – the next step!

What is ”future” and what is ”rapid”?

- In AST the future has been around the corner since I took up clinical microbiology in 1971.
  Rapid ID (Maldi-Tof) and harmonisation of breakpoints are the most prominent achievments.
  And for some of us, the future is shrinking rapidly.

- ”Rapid” could be defined as having an ID- and AST-result within one hour of receiving the sample.

- ”Slow” could be defined as having an ID + AST 40 - 48 hours after receiving the sample. This is often today´s (and yesterday´s) standard!

Methods for susceptibility testing

- **Phenotypic test methods**
  based on **antimicrobial activity (MIC)** and **breakpoints (S≤X/R>Y mg/L)**

- **Genotypic test methods**
  based on the detection of a **resistance gene** or its **product**

- **Deduction** – ”expert rules”

Methods for susceptibility testing

- **Phenotypic test methods**
  based on **antimicrobial activity (MIC)** and **breakpoints (S≤X/R>Y mg/L)**
  - MIC, disk diffusion, automated systems like Phoenix, Vitek2, Microscan
  - **Predicts susceptibility and resistance**
  - **Quantifiable**
- **Genotypic test methods**
  based on the detection of a **resistance gene** or its **product**
  - mecA, vanA, vanB, ….PBP2, … betalactamase detection….
  - **Predicts resistance, not sensitivity**
  - **Not quantifiable**
- **Deduction** – ”expert rules”
  - If mecA-positive then report betalactam antibiotics R;
    If ESBL-positive, then report betalactam antibiotics R – or maybe not!?
    If erythromycin-resistant, then report roxithro- and clarithromycin R;
  - **Some rules predict susceptibility, others resistance.**
  - **Rules change over time**
  - **Not quantifiable**

Limitations of current methods?

- Phenotypic methods:
  - Require **breakpoints** – which calls for agreement!
  - Current methods normally 16 – 20 hours
  - Require species ID
  - Semi-automated or not automated.
- Genotypic methods:
  - Predict resistance – not susceptibility.
  - Predict known resistance – not new resistance.
  - Requires species ID
  - Not fully automated

EUCAST was formed in 1996 and reformed in 2001.

Committee	Country	Disk Diffusion test?
EUCAST 	Europe	Yes
CLSI 	USA	Yes

*EUCAST is now an umbrella for national breakpoint committees BSAC, CA-SFM, CRG, DIN, NWGA & SRGA.

EUCAST and the review process

Reviewed 2002 – 2009:

- Aminoglycosides
- Carbapenems & aztreonam (2nd review)
- Cephalosporins iv (2nd review)
- Cephalosporins oral
- Fluoroquinolones
- Glycopetides (2nd review)
- Macrolides and lincosamines
- Miscellaneous antimicrobials
- Penicillins
- Tetracyclines

- Antifungal drugs
  - fluconazole, voriconzole, posaconazole
  - anidulafungin, amfotericin B

Topical agents:

Mupirocin (LLR/HLR)
Retapamulin (ECOFF)

Drugs being addressed:

Cefalothin (ECOFF)
Cefazoline (ECOFF)
Cefoperazone (ECOFF)
Sulbactam (alone)
Kanamycin
Streptomycin
Josamycin
Spiramycin
….

Lack of data for older drugs!

EUCAST
- breakpoints for new drugs with EMA

- Daptomycin √
- Tigecycline √
- Doripenem √
- Glycopeptides (two ongoing)
- Cefalosporine (ongoing)
- Anti-Tb (to be started)

- Glycopeptide (withdrawn)
- Cefalosporine (withdrawn)
- Fluoroquinolone (withdrawn)
- Diaminopyrimidine (withdrawn)

- Extensions of indications (currently none)

EMA = European Medicines Agency

Miscellaneous organisms

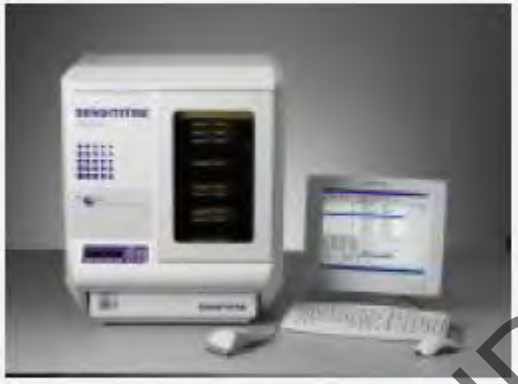
Consultation with expert groups on breakpoints and methods

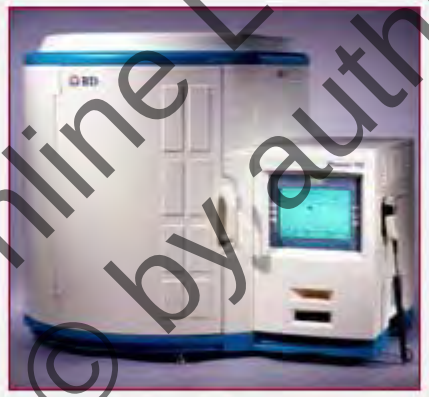
- Neisseria meningitidis (review) - 2011
- Moraxella catarrhalis (revised) - 2011
- Helicobacter pylori (finalized) - 2011
- Clostridium difficile (finalized) - 2011
- Campylobacter (ongoing) - 2011
- Listeria monocytogenes (consult) - 2011
- Pasteurella multocida (ongoing) - 2011
- Burkholderia cepacia (started) - 2012
- Corynebacteria (ongoing) - 2012
- M.tuberculosis – new agents - 2011/12
- …

Common practices

- **Standardised phenotypic AST 16 – 20 hours**
  - EUCAST or CLSI or BSAC or CA-SFM.
- **”Direct” testing** – shaves 20-24 h off the standard practice of culture (20h) + AST (16 – 20h).
  - Urine – direct inoculation
    - Of all urines
    - Of positive urines (microscopi, rapid tests, flowcytometri)
  - Blood cultures – direct (or semidirect) inoculation from a positive bottle
- **”Rapid AST”** with automated methods
  - 8 h from pure culture
- **”Rapid AST”** with MIC- or Disk Diffusion tests
  - Results 8 h from pure culture
  - Results 8 h from ……..?????

MIC based automatic systems





Automation of AST – to…

- Increase speed – rapid AST reports
- Reduce need for hands-on work
- Reduce the need for know how
  - In running the test
  - In interpreting the result
- Increase standardisation
- Simplify quality control
- Reduce costs

…will current trends in automation deal with the future?

Very few hits on:

- ”Automation in AST” - except evaluations of Phoenix, Vitek2, Microscan
- ”Rapid methods in AST” – basically a plethora of gene detection protocols – some commercially available, others not (NDM-1, CTXinases, mecA and on variations to cover new genes, etc)
  - Maldi-Tof – for the detection of ESBLs etc ??
  - Flowcytometri, microcalorimeter detection of growth ??

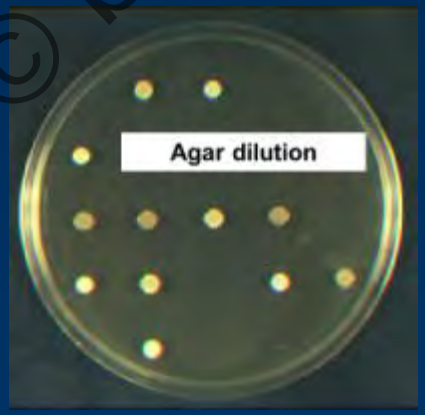
MIC

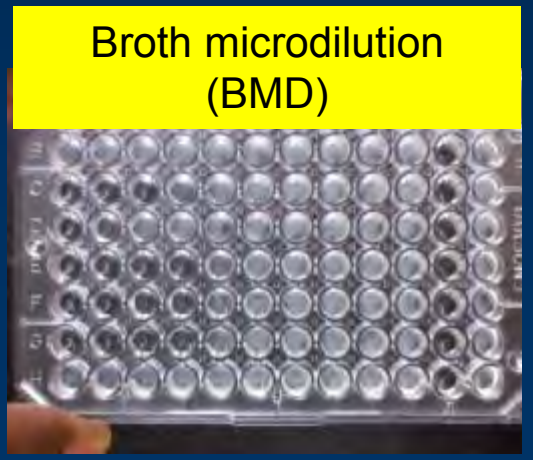
- MIC – the minimum inhibitory concentration (mg/L or µg/mL)
- MIC - the lowest concentration in a series of twofold concentrations that will inhibit the growth of a microorganism, as measured by the naked eye.
- Convention: The concentration series shall contain the concentration 1 mg/L

  0.002, 0.004, 0.008, 0.015, 0.03, 0.06, 0.12, 0.25, 0.5, 1, 2, 4, 8, 16, 32, 64, 128, 256, 512

Methods for antimicrobial susceptibility testing
MIC

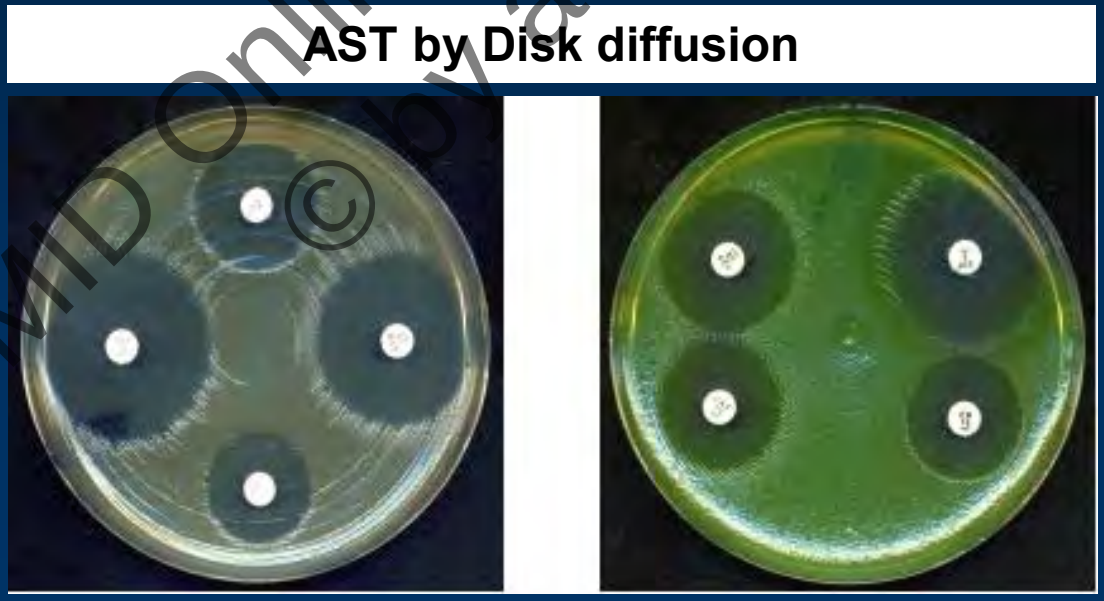
- Broth dilution
- Broth microdilution (BMD)
- Agar dilution
- Gradient tests (several)

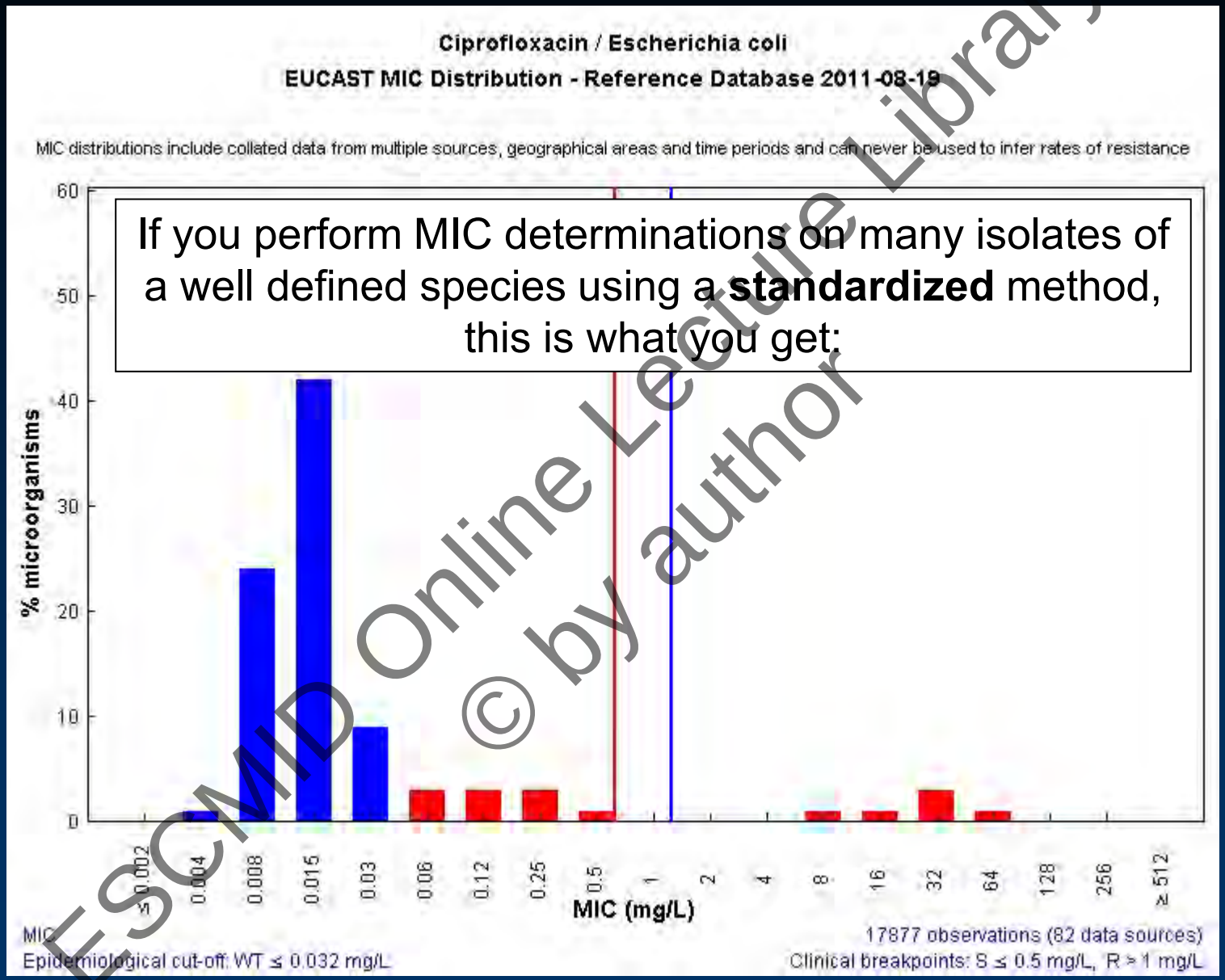


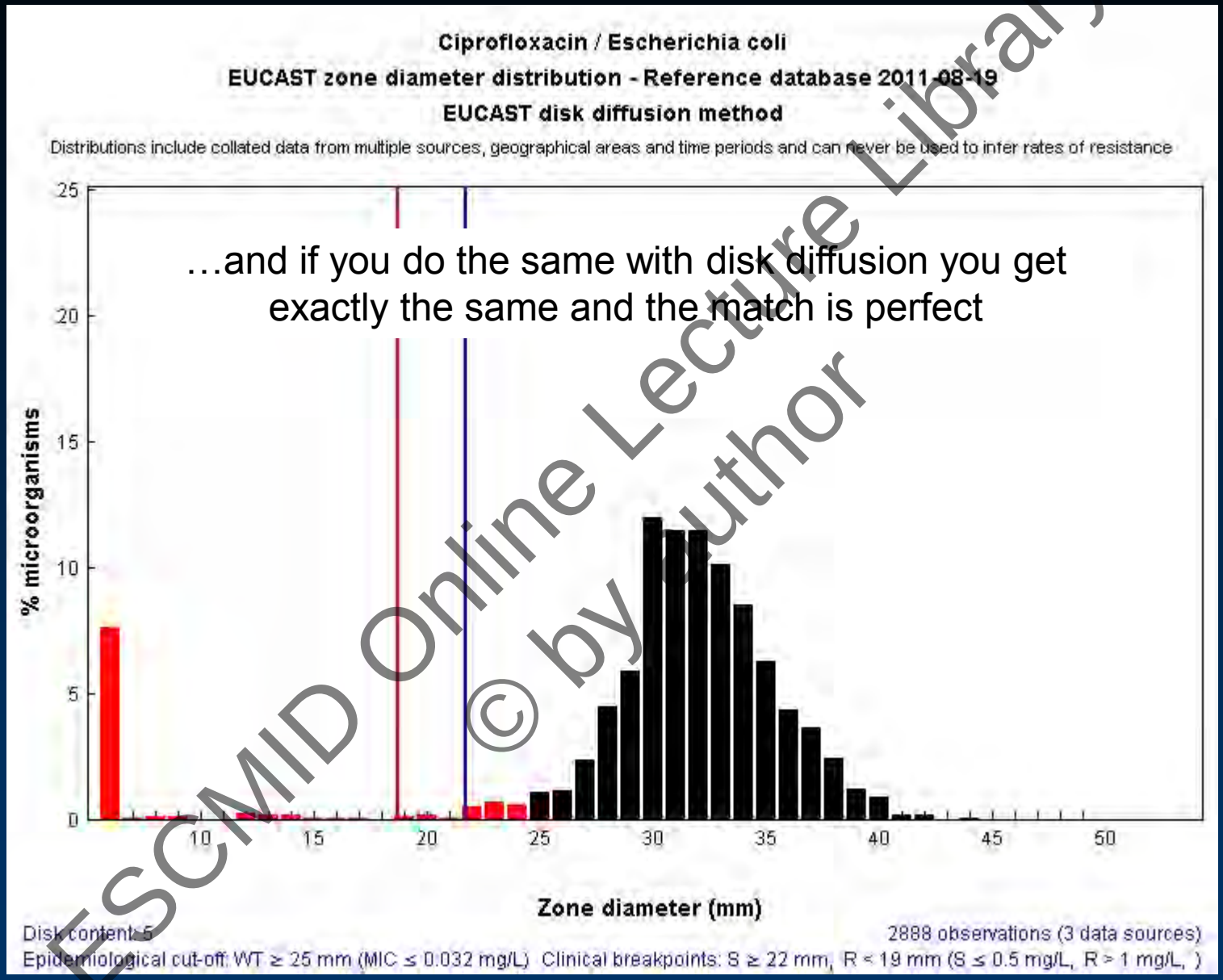


Methods for antimicrobial susceptibility testing
Disk diffusion (DD)

- Disk diffusion
  - Correlated to MIC
  - Standardised DD: CLSI, EUCAST, BSAC, CA-SFM
  - MH (CLSI, EUCAST, CA-SFM), ISA (BSAC)

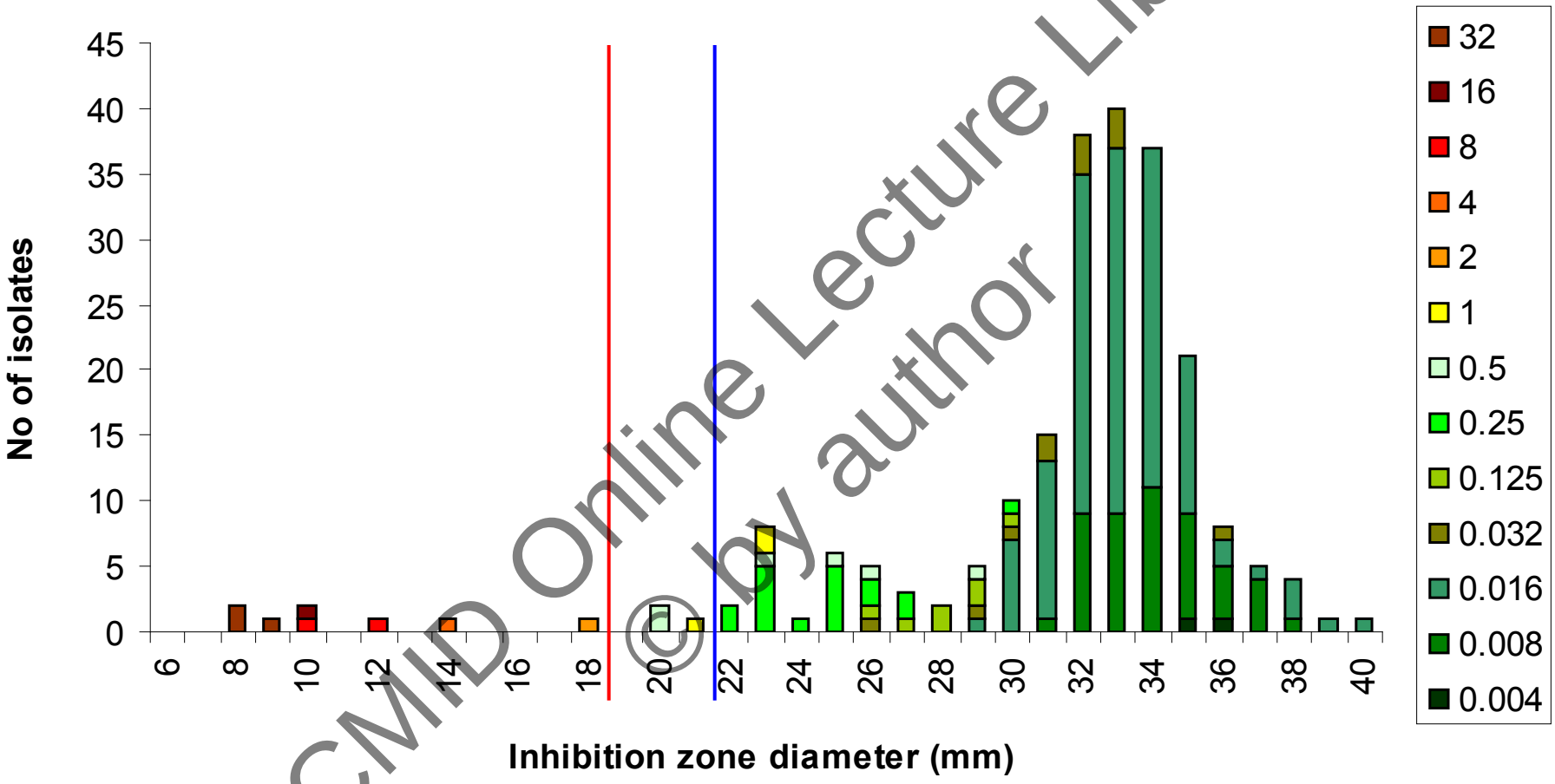






E. coli with ciprofloxacin 5 ug

223 clinical isolates



Breakpoints	S	R
MIC	≤0.5	>1
Zone diameter	≥22	<19

Automated systems

- **MIC-based (semiautomated)**
  - Microdilution (Phoenix, Microscan, Sensititre)
  - Growth curve (Vitek2)

- **Disk Diffusion (semiautomated)**
  - Biomic, Sirscan, Osiris

Automated AST typically offer

- Interface with LIS (Laboratory Information Systems)
- Quality Control programs
- Interpretative reading and Expert Rules:
  - Intrinsic susceptibility (warn against reporting resistant)
  - Intrinsic resistance (warn against reporting susceptible)
  - Expert rules: IF – THEN (deduce susceptibility and resistance)

- Resistance epidemiology features

Essential agreement

- ...can not be assessed when dilution series are truncated

Expected	.06	.12	.25	.5	1	2	4	8	16
Obtained			≤.25	.5	1	2	>2		
MICs (n)	24	52	31	3	2	22	31	12	45

Categorical agreement (CA)

- Number of categorical results (S, I and R) in agreement

Agreement	Expected/Obtained
CA	S/S
CA	I/I
CA	R/R
minor errors	S/I, I/S, I/R, R/I
ME	S/R (false resistance)
VME	**R/S** (false susceptibility)

Automated AST

Pros

- Combined AST and ID – but are we interested?
- Speed (6, 8 – 18h)
- Labour – saving - ??
- Cost – savings - ??
  - Counting exceptions and limited capacity and the need for running a supplementary test, this needs careful consideration.
- Standardisation
- QC
- Expert rules to suggest resistance mechanism.

Automated AST

Cons

- Many exceptions – supplementary testing needed.
  - Missing agents
  - Missing organisms
- Do not respond well to rapid development
  - New drugs
  - New breakpoints
  - New resistance mechanisms
  - **Approval procedures take too long**
  - Updates take too long
- Limited capacity
- Limitations caused by short dilutions series
- Limitations caused by availability of panels

Approval process
The process is too time consuming!

- The machines are ”susceptibility testing machines” and are approved as such.
- Performance and approval focus on categorical agreement.
- Every breakpoint change needs new FDA approval.

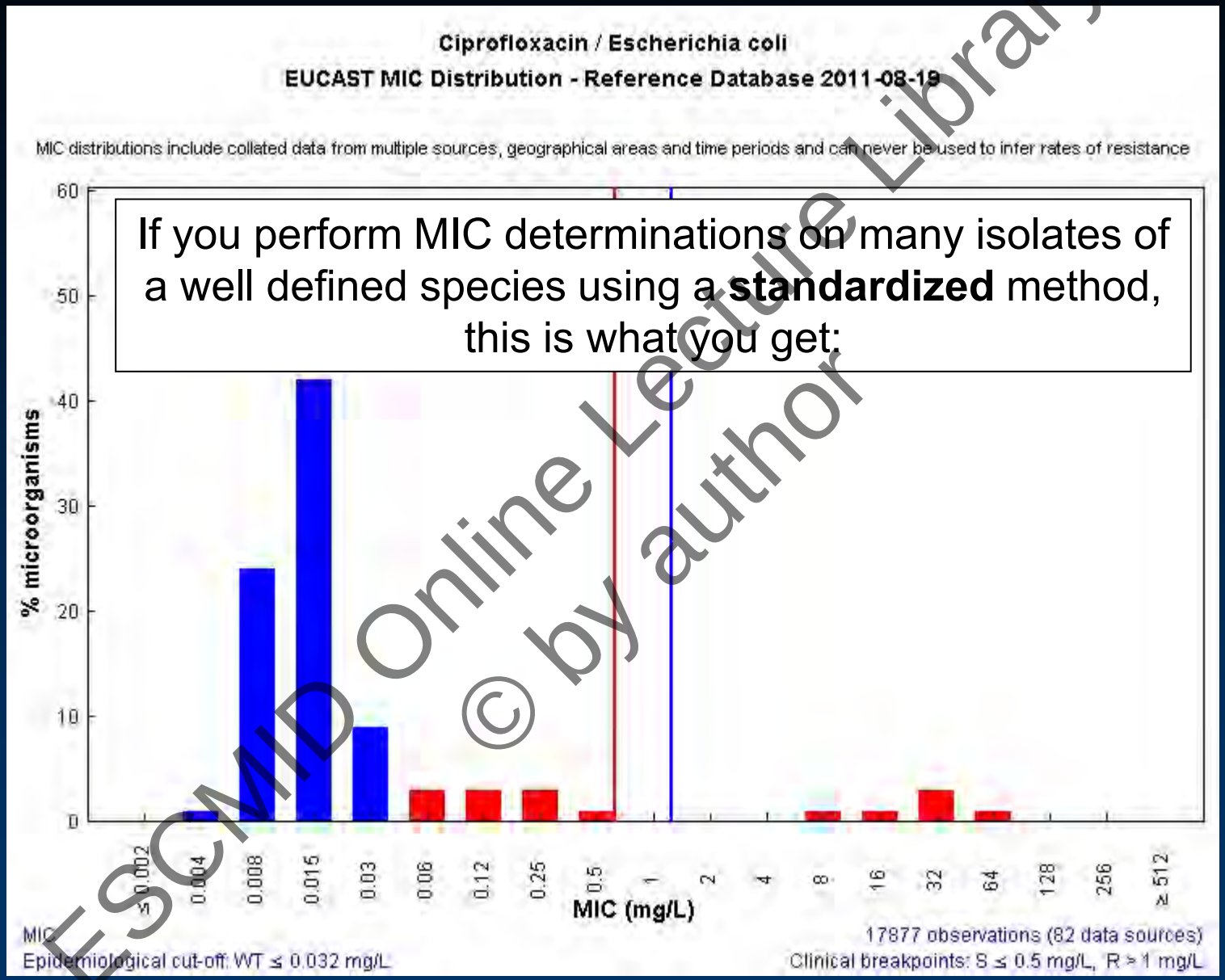
- The machines should have been developed as ”MIC determination machines”:
- Approval should have been focused on essential agreement only – ”get the MIC right”.
- ….in which case breakpoint changes could be implemented without new FDA approval.

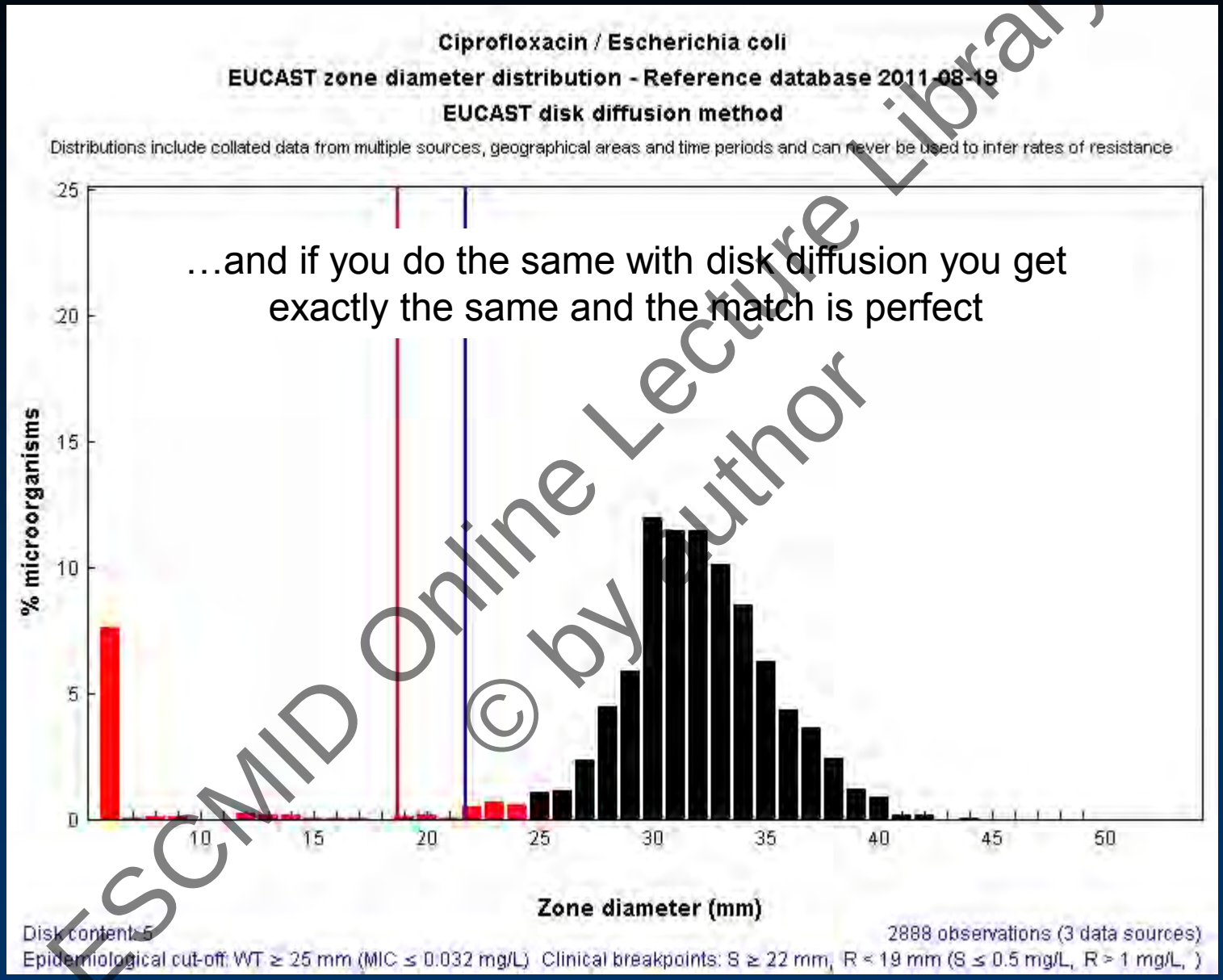
Future phenotypic AST (6 - 8 h)

- Automated **MIC-determination**
  - ID known through Maldi-Tof
  - machines dedicated to MIC determination
  - complete dilution series
  - approval on MIC performance (not on categorical agreement)
  - 1 – 4 - 6 – 8 h (!?) – new techniques for measuring growth may reduce time further.
  - Output: WT or NWT, Clinical S, I o R

Future phenotypic AST (6 – 8 h)

- Automated **disk diffusion testing**
  - Automated
    - plate inoculation,
    - disk application,
    - incubation
    - and zone diameter reading against variable protocols. Database of zone diameter distributions related to incubation time and adjusted breakpoints.
  - Output: WT or NWT, Clinical S, I or R





Future susceptibility report?

Clinical breakpoints and assessment of resistance mechanisms

- Escherichia coli
  - Ampicillin R
  - Cefotaxime S^{NWT}
  - Trimethoprim R
  - Nitrofurantoin S
  - Ciprofloxacin S^{NWT}
- Enterococcus faecalis
  - Ampicillin S
  - Gentamicin R^{WT}
  - Ciprofloxacin IE^{WT}

Back to the beginning!

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The Future

- **Any new techniques on the horizon?**
  - Maldi-Tof,
  - Alternative ways of measuring growth for early phenotypic detection of R?
- **Any promising ”rapid” methods around?**
  - Automatic reading of disk diffusion? **8 hour AST**
  - There should have been an MIC-determination machine around but alas!
- **Global harmonisation of breakpoints?**
  - CLSI and EUCAST getting ready to harmonize? NO
  - EUCAST is strong and making headway in many countries!
- **Global harmonisation of methods?**
  - Yes – to some extent.
- **TATFAR (transatlantic taskforce on AR) – progress?**
  - CDC and ECDC have been tasked with organising common ECOFFs!
- **EUCAST beyond 2011?**

EUCAST and the future

- 2012 new contract with ECDC
- External expert committee financed by ECDC and peopled by the profession
- Developing standards for ECDC surveillance (AMR, HCAI)
- The breakpoint committee of EMA for new, including new anti-Tb agents, and existing antimicrobials
  - breakpoints and ECOFFs.
- Standardizing AST methods and IQC and advising on EQC.
- Communicating with national AST committees (NAC) and the profession through public consultation and the website (www.eucast.org)
- Education
- Address issues with locally acting agents and with the activity of agents i biofilms.

Thank you