UNIVERSITY OF CAMBRIDGE

# The role of whole genome sequencing in antimicrobial susceptibility testing of bacteria: report from the EUCAST Subcommittee

Clin Microbiol Infect. 23(1):2-22 (2017)

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# Disclaimer

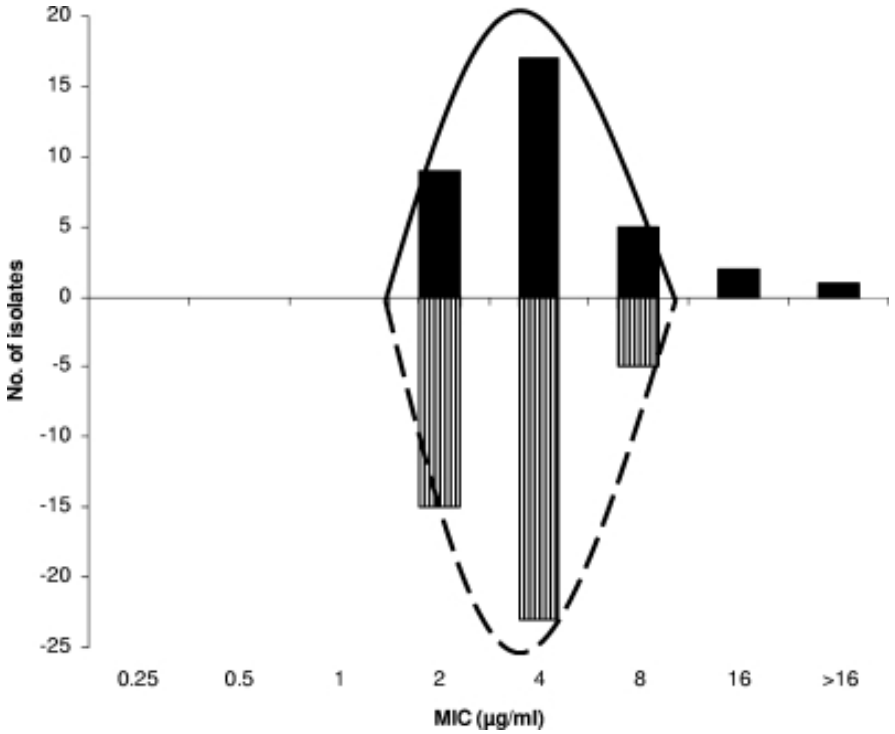
- I am consultant for the Foundation for Innovative New Diagnostics (FIND).
- The Bill & Melinda Gates Foundation, Janssen Pharmaceutica, and PerkinElmer covered my travel expenses to present at meetings.
- I have been awarded the Gertrud Meissner Award, which is sponsored by Hain Lifescience.
- I have collaborated with Illumina on a number of scientific projects.

# Drug susceptibility testing

- Resistance traditionally defined phenotypically with no (or little) understanding of resistance mechanisms.
- However, phenotypic DST is far from being free of assumptions.

# What is the reproducibility of MIC testing?

- ISO 20776-2: within ± 1 dilution of the mode of that antimicrobial agent for ≥95 % of the results




Fluconazole MIC distributions of 34 individual *C. glabrata* isolates (black bars) compared with the MICs obtained by 51 repeated tests of a single *C. glabrata* isolate (striped bars) originally determined to have a MIC of 2 µg/ml.

ISO 20776-2

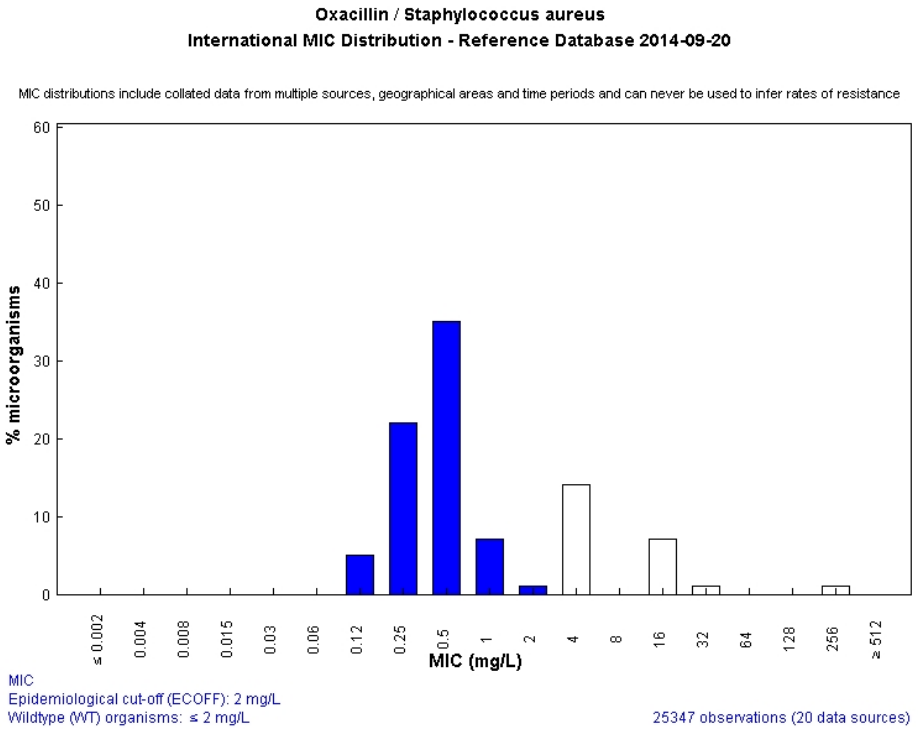
Arendrup et al. Antimicrob Agents Chemother. 2009 Apr;53(4):1628-9

# ECOFF

- Epidemiological cut-off value (ECOFF): Also known as microbiological breakpoint. It corresponds to the highest concentration/upper end of the wild-type (WT) MIC distribution (i.e. Gaussian distribution with organisms devoid of **phenotypically detectable resistance**).

Kahlmeter J Antimicrob Chemother. 2015 Sep;70(9):2427-39

# ECOFF




- Conditions for acceptance of data by EUCAST:
  - Minimum number of isolates and laboratories
  - WT distribution not truncated
  - Peaks of distributions need to be similar
- ECOFF can be defined ‘by eye’ or using a variety of statistical methods.

Kahlmeter J Antimicrob Chemother. 2015 Sep;70(9):2427-39

EUCAST

EUROPEAN COMMITTEE ON ANTIMICROBIAL SUSCEPTIBILITY TESTING

European Society of Clinical Microbiology and Infectious Diseases

# EUCAST Subcommittee on MIC distributions and epidemiological cut-off values (ECOFFs)

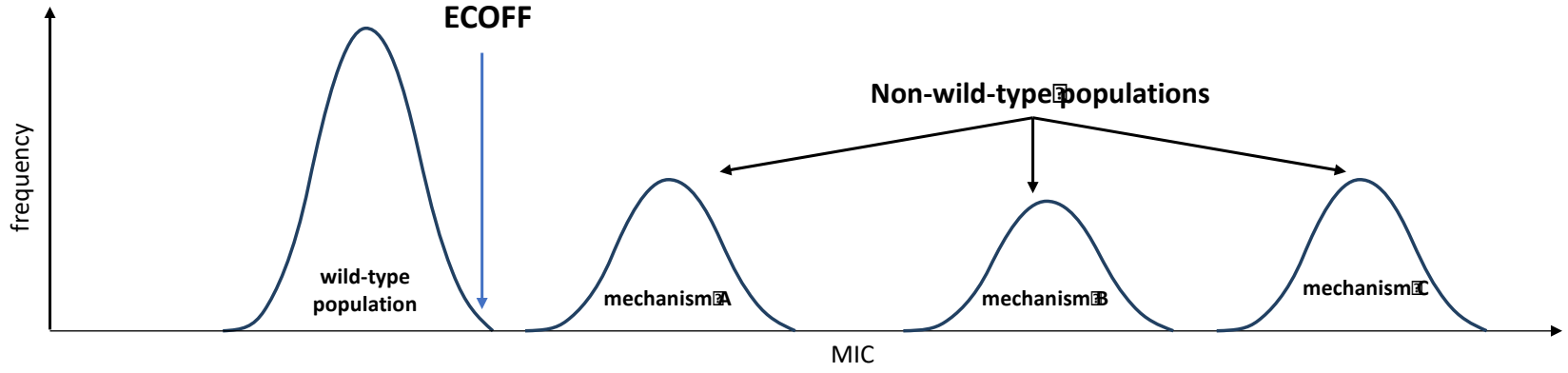
**Discussion document Version 3, 7 March 2017**
**For general consultation 9 March – 14 May.**

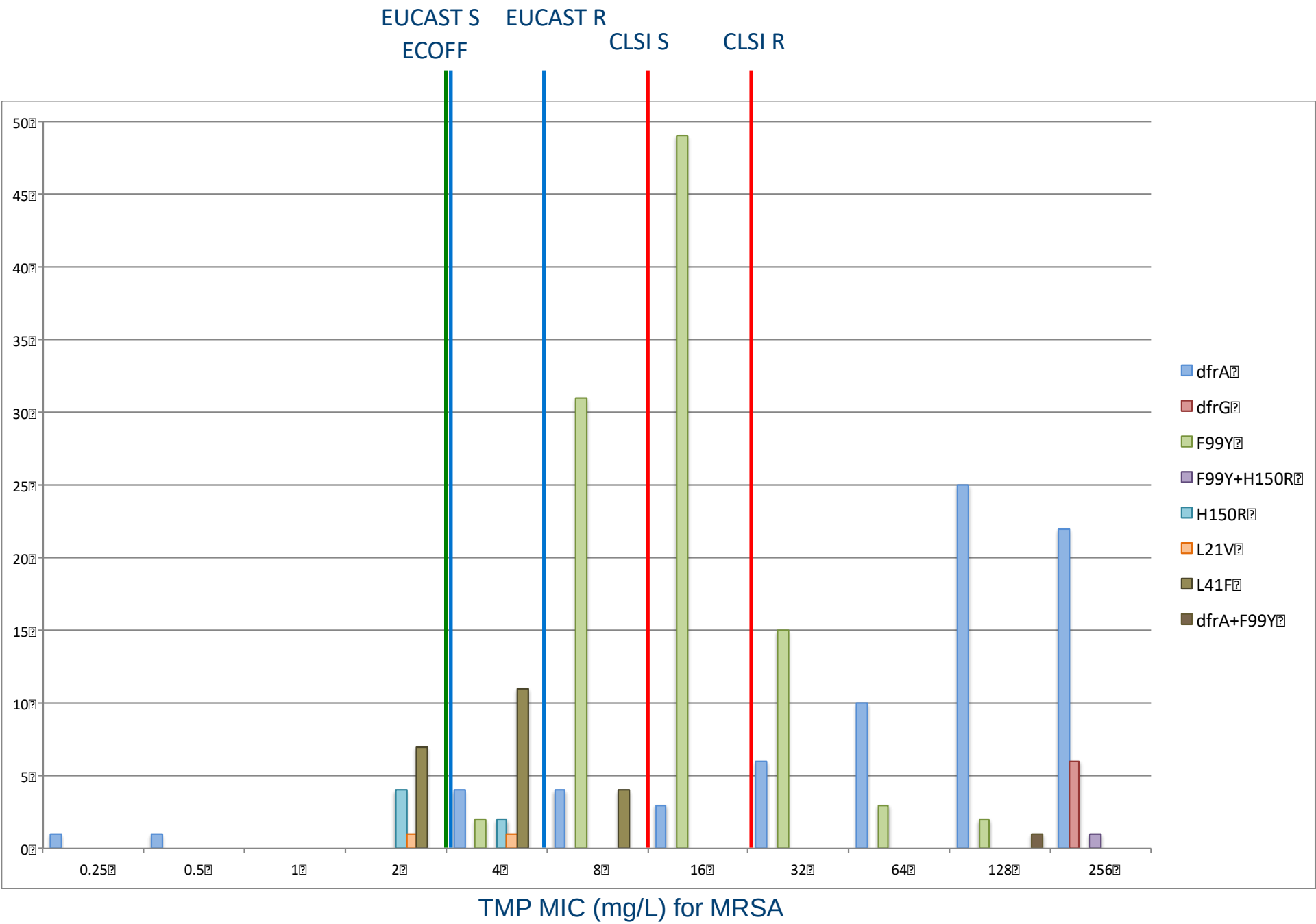
# ECOFF vs. CB

- Epidemiological cut-off value (ECOFF): Also known as microbiological breakpoint. It corresponds to the highest concentration/upper end of the WT MIC distribution (i.e. Gaussian distribution with organisms devoid of phenotypically detectable resistance).
- Clinical breakpoint (CB): MIC value that distinguishes organisms of the same species (or closely related species) that are likely to succeed therapy from those that are likely to fail (i.e. S/I/R system). CB can differ between EUCAST, CLSI, FDA, and WHO.
- CB should be ≥ ECOFF (except if an organism is intrinsically resistant and other rare exceptions where this rule has not been implemented, yet)

Kahlmeter J Antimicrob Chemother. 2015 Sep;70(9):2427-39

Setting MIC data alone




EUCAST S
ECOFF
EUCAST R
CLSI S
CLSI R
50
45
40
35
30
25
20
15
10
5
0
0.25
0.5
1
2
4
8
16
32
64
128
256
dfrA
dfrG
F99Y
F99Y+H150R
H150R
L21V
L41F
dfrA+F99Y
TMP MIC (mg/L) for MRSA

# Defining resistance for TB

- Guiding philosophy:
  - Full transparency for accountability
  - Clear documentation of assumptions and open questions to foster research
- Approach for each drug:
  - How reproducible are distributions between laboratories?
  - What is the WT distribution?
  - What do we know about the NWT distribution(s) and the underlying resistance mechanism(s)?
  - How close are WT and NWT distributions? Is it possible to set an optimal ECOFF/CB?
  - Review of PK/PD data to investigate whether current dosing is appropriate for WT distribution and whether, if any, NWT strains are still treatable at standard or increased dose
  - Role for expert rules (i.e. should genotype overrule phenotype in some cases)?

# Summary

- In theory, phenotypic DST should be superior to genotypic alternatives, but phenotype is far from being free of assumptions
- Unlike for most pathogens and antibiotics, the WT and NWT distributions for TB are very close or even overlapping for some antibiotics, which means that phenotypic DST has poor reproducibility
- In these circumstances, genotypic DST can be a better way to detect resistance caused by known resistance mechanisms
- We have to acknowledge and tackle this ‘messiness’ to stem the tide drug-resistant TB

# Thank you very much for your attention