Overall outcomes of the EURL-AR EQAS 2019 for *Escherichia coli*, *Staphylococcus aureus* and *Enterococcus* sp.

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What is evaluated in this EQAS

- AST of 8 *E. coli*, 8 *Enterococcus* sp. and 8 *S. aureus* test strains
  - MIC determination
  - Interpretation according to EUCAST ECOFFs

- Detection of resistance phenotypes of particular public health relevance
  - ESBL/AmpC/carbapenemase production in *E. coli*
  - MRSA

- *Enterococcus* species identification

- Test of ATCC strains for QC

Evaluation = presence/absence of deviations

Deviations: let's set the record straight

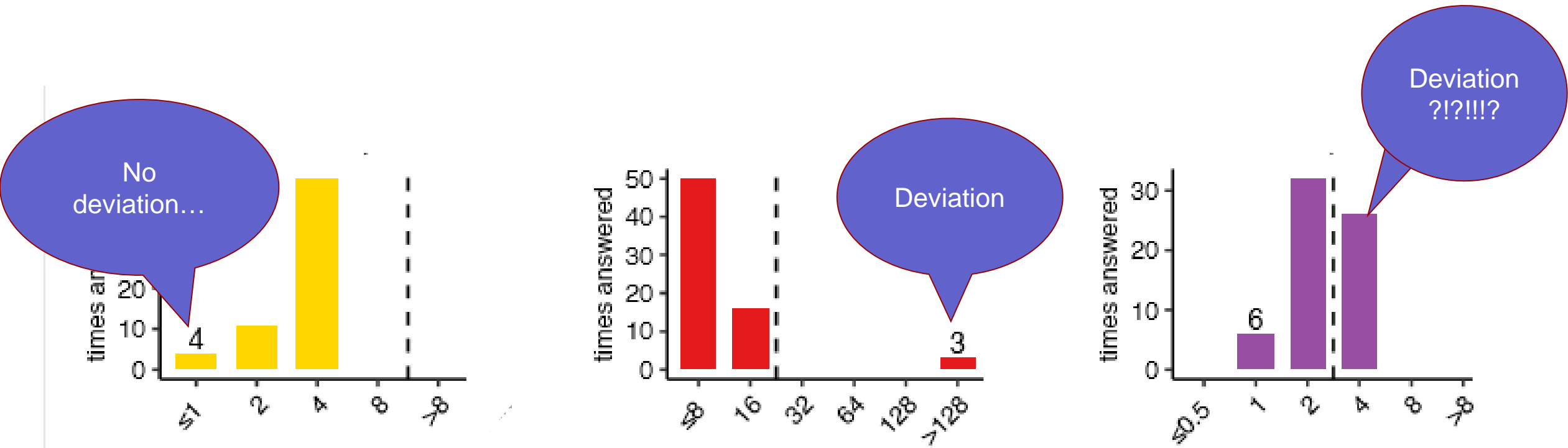A **deviation** is due to obtained <u>interpretation</u> different from the expected interpretation. But…

1. We interpret MIC values

2. When performing broth microdilution, the 'right' MIC is indeed a range of values due to limitations in reproducibility of the method (*see very interesting papers by Johan Mouton – very good reading in the quarantine period!*)
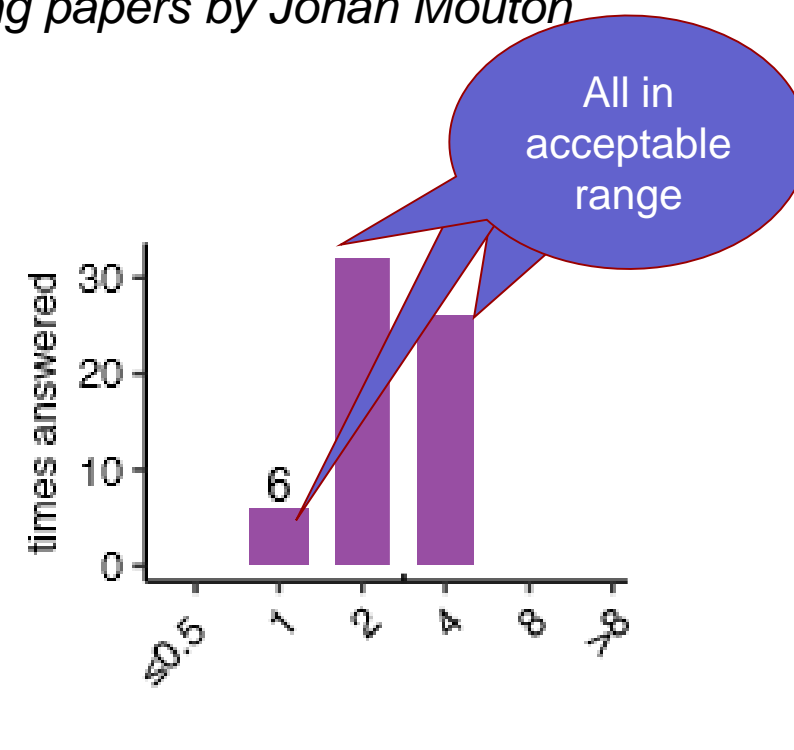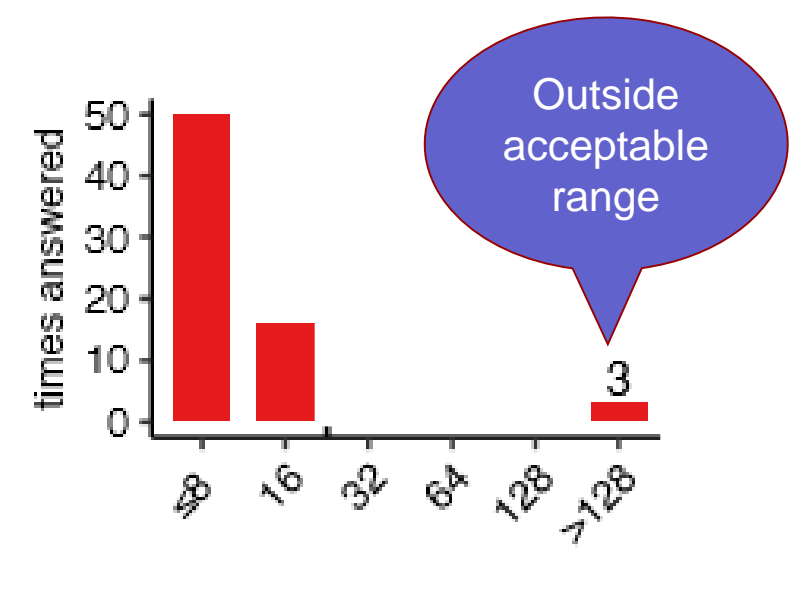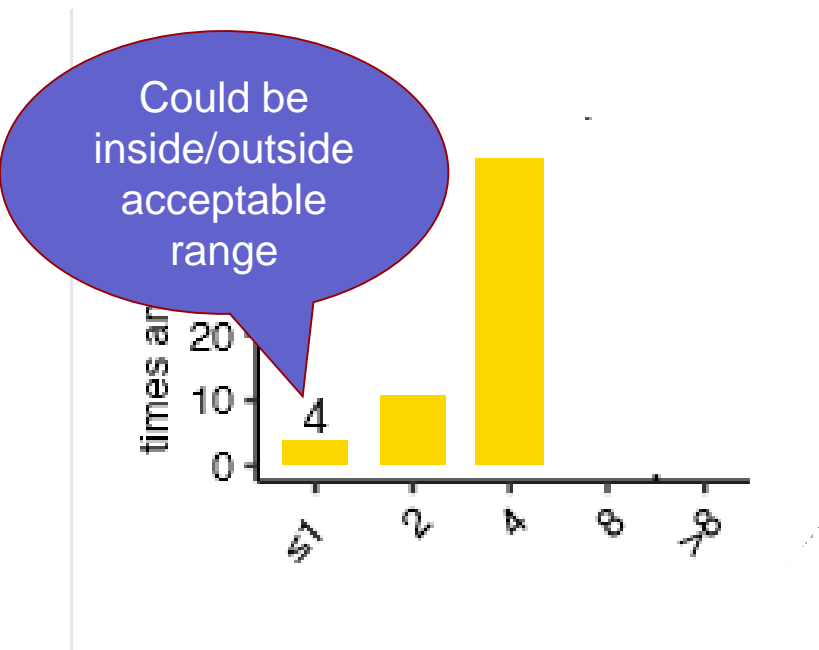
Deviations: let's set the record straight

If the 'right' MIC is close to the ECOFF (dotted vertical line in the figures), then different interpretations will be obtained – and one of them will be scored as a deviaton - for MIC values which are otherwise in the acceptable range

Sometimes, a MIC obtained in the acceptable range is **interpreted erroneously**… distraction issue, easy to overcome or use of different interpretive criteria…

These deviations might indicate a **technical problem** in obtaining MIC in the acceptable range (or distraction in uploading results…)

Such deviations cannot be corrected – it is not the operator's fault but the limitation of the method. Thus, I call them **"one-fold dilution issues"**
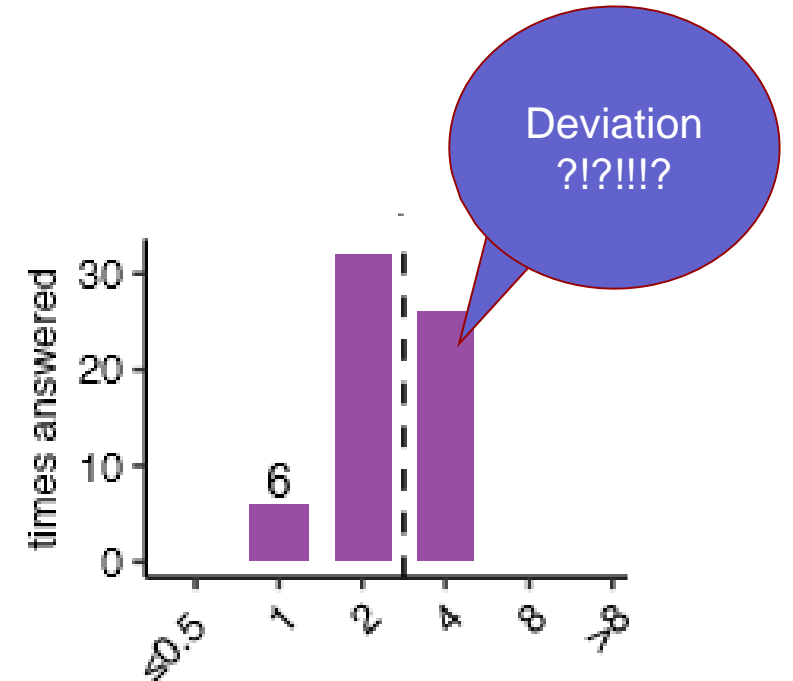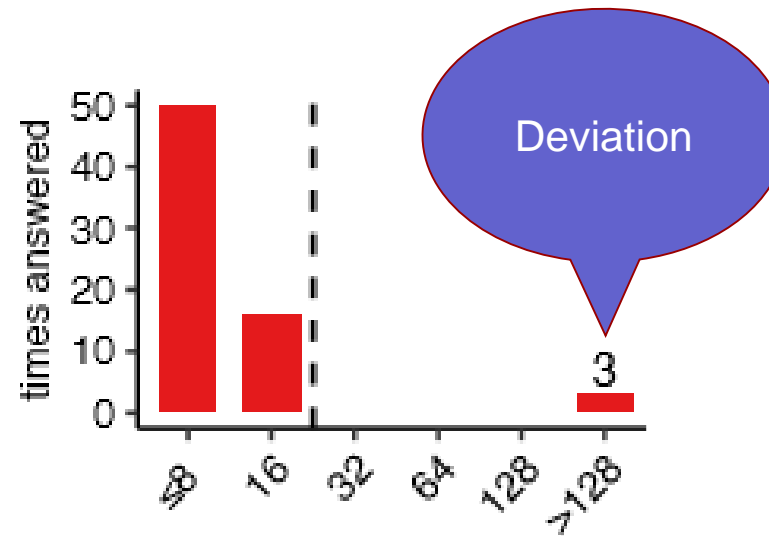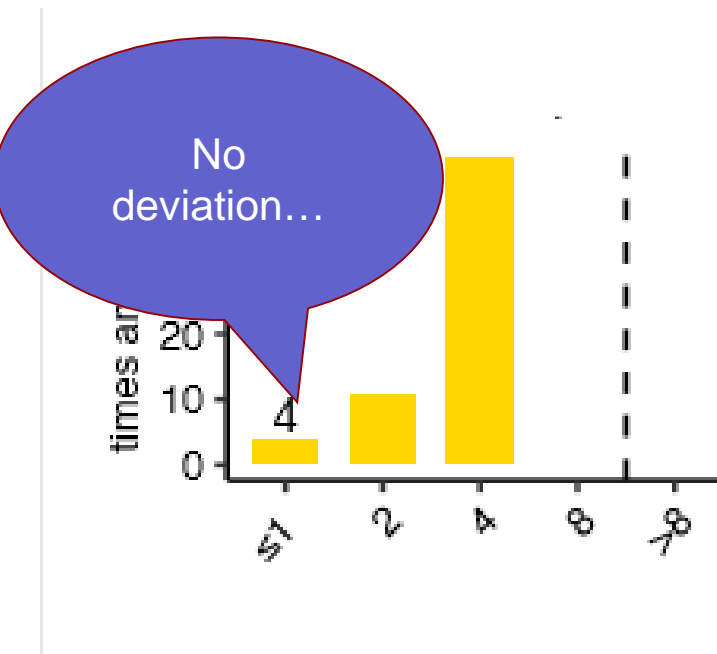
The annoying "one-fold dilution issues"

- Due to the "one-fold dilution issues", the network agreed many years ago to remove from the report the strain/antimicrobial combinations for which there are > 25% deviations

- I often receive the question if searching for antimicrobial resistance genes/mutations would give the final answer in setting the 'right' MIC. The answer is **no** because:

  – We interpret MIC values using ECOFFs which, by definition, are based on MIC distributions without taking into consideration the presence/absence of resistance genes. Usually presence/absence of resistance genes correlates well with non-wild-type/wild-type (which we call resistant/susceptible in the report, for convenience), respectively, but it is a consequence and not a reason for the classification
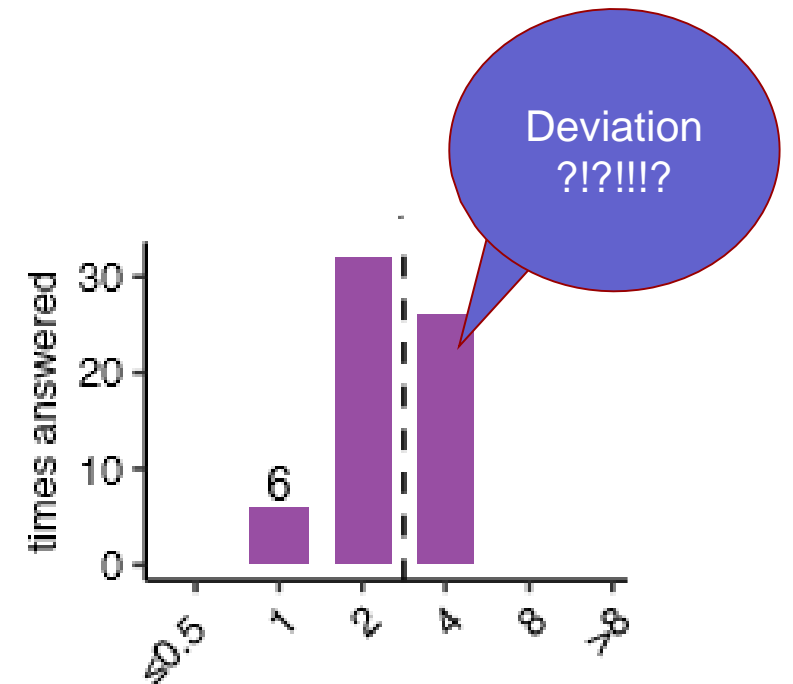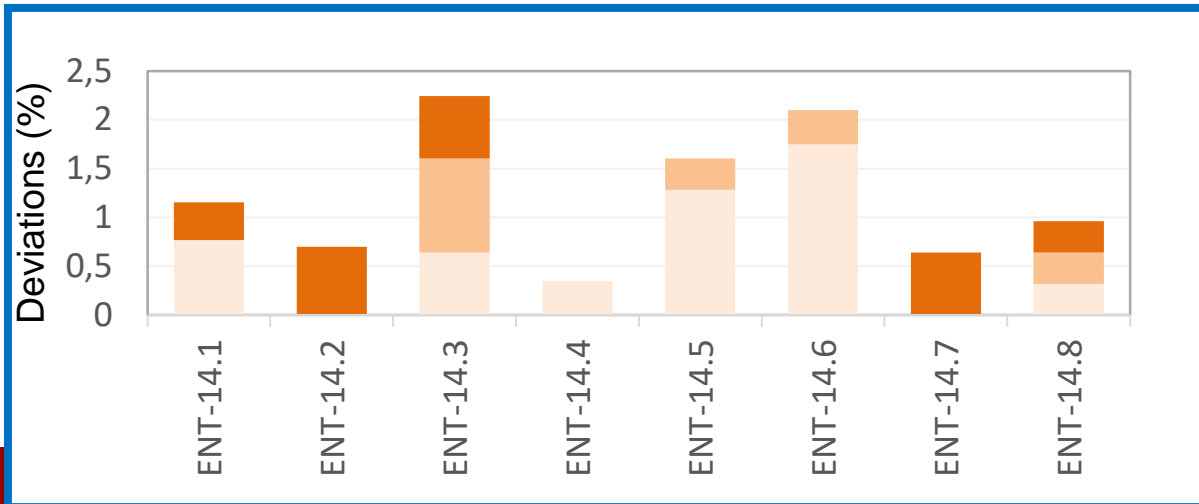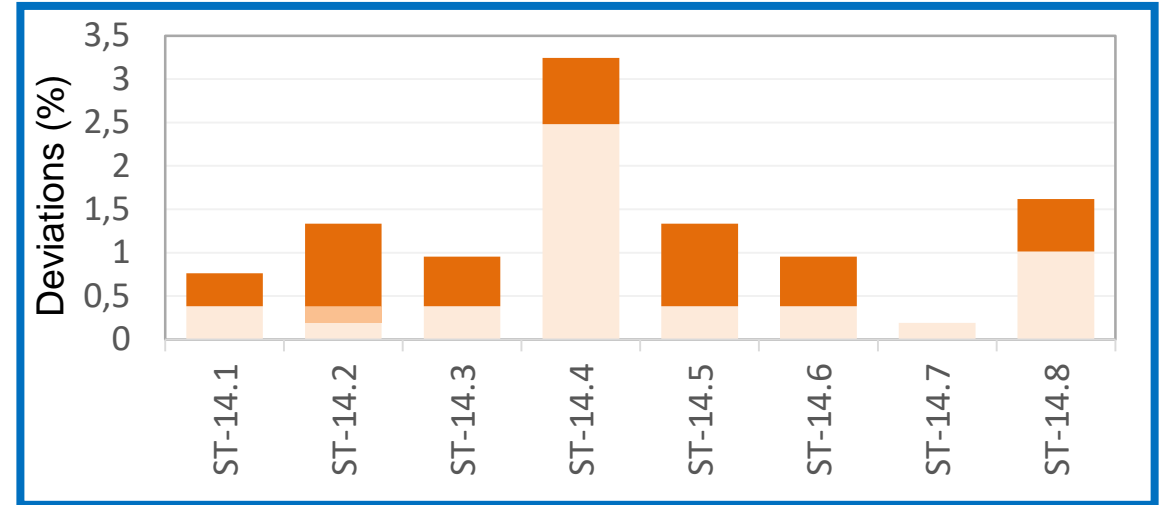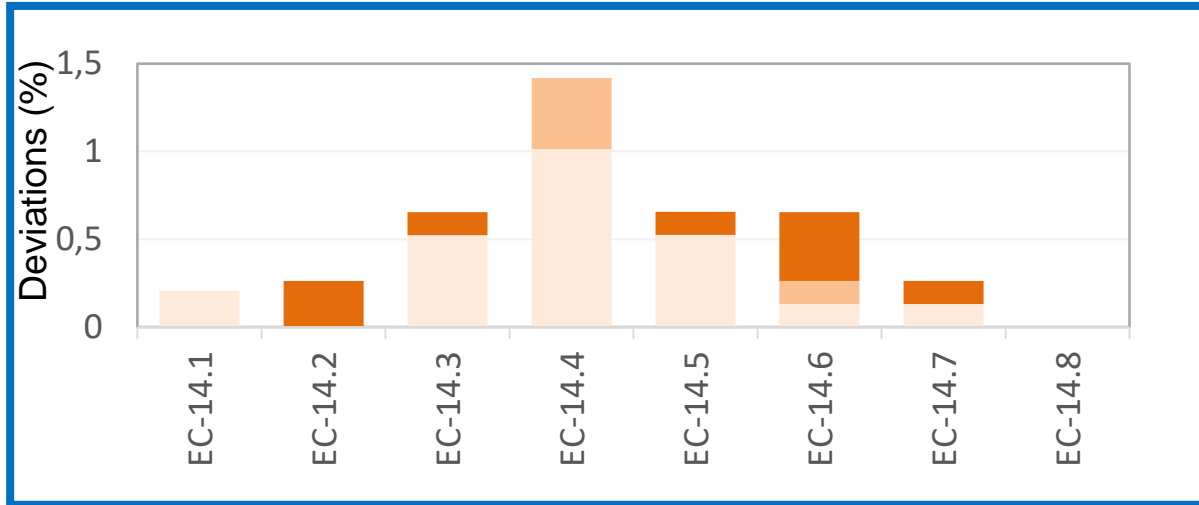
  – The concept of 'right' MIC is flawed

Such deviations cannot be corrected – it is <u>not</u> the operator's fault but the limitation of the method. Thus, I call them **"one-fold dilution issues"**

Overview of the strains:
did you find any nasty strain?

After removing a few strain/antimicrobial combinations for which there were > 25% deviations

MIC one-step dil. from expected value

MIC as expected but interpreted differently

MIC outside acceptable range

Overview of the antimicrobials: what's your favorite drug?

E. coli

Overview of the antimicrobials: what's your favorite drug?

Enterococcus sp.
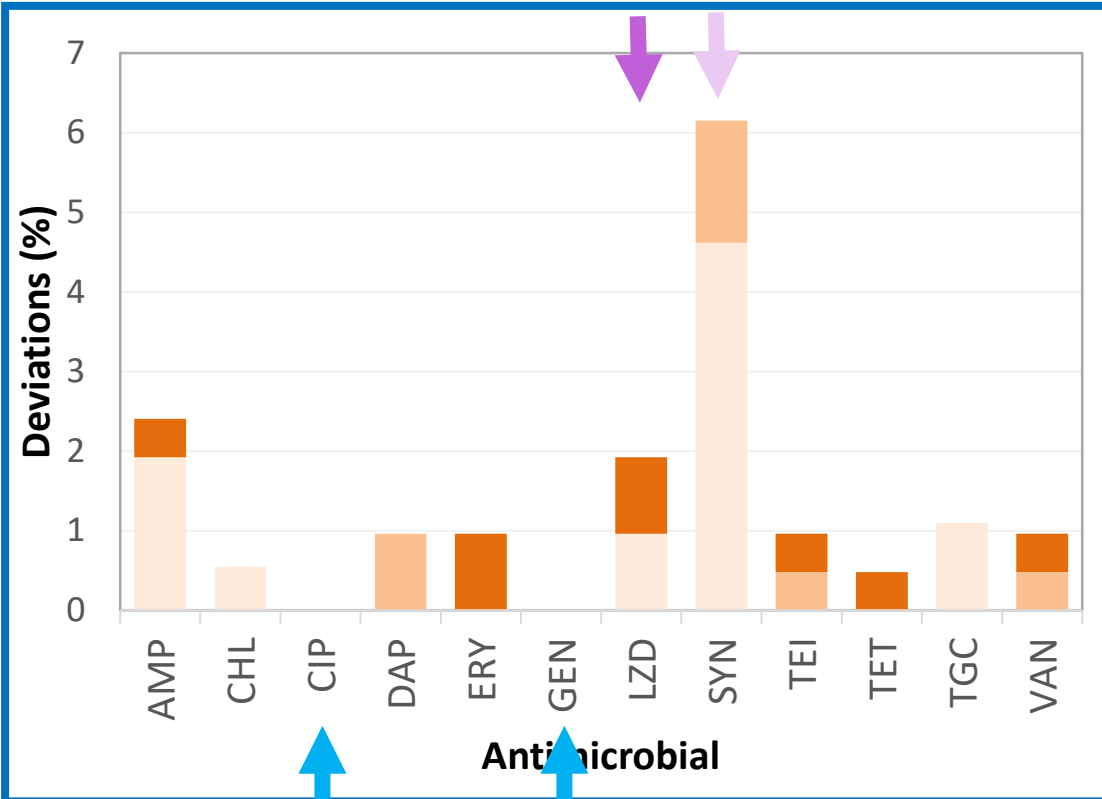
Staphylococcus aureus
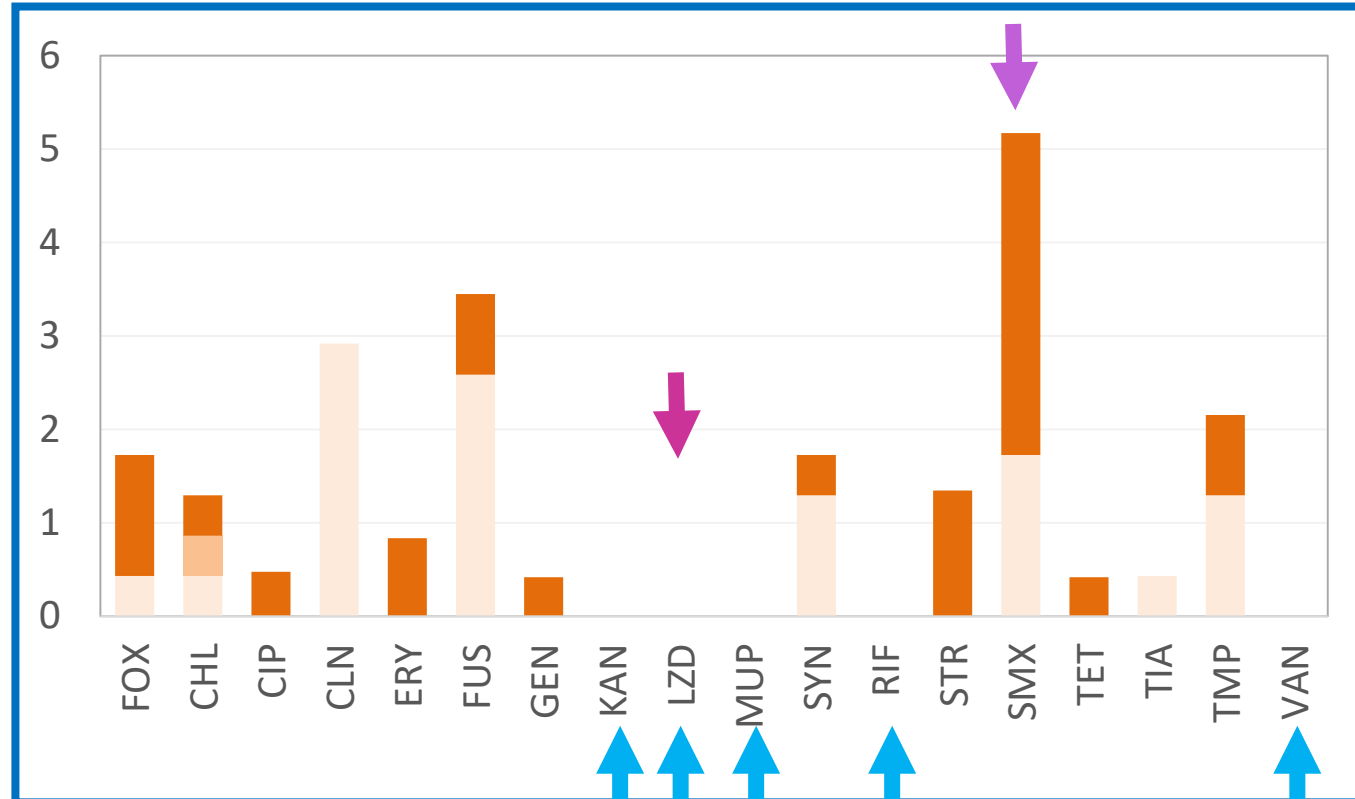
Legend:
- MIC outside acceptable range
- MIC as expected but interpreted differently
- MIC one-step dil. from expected value
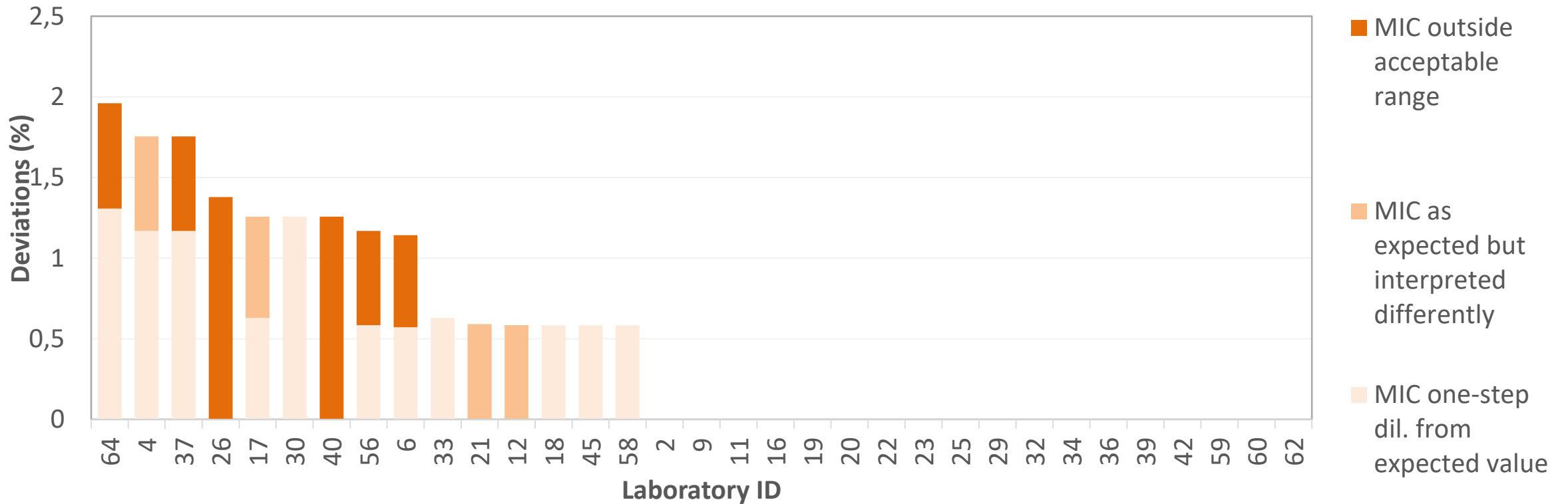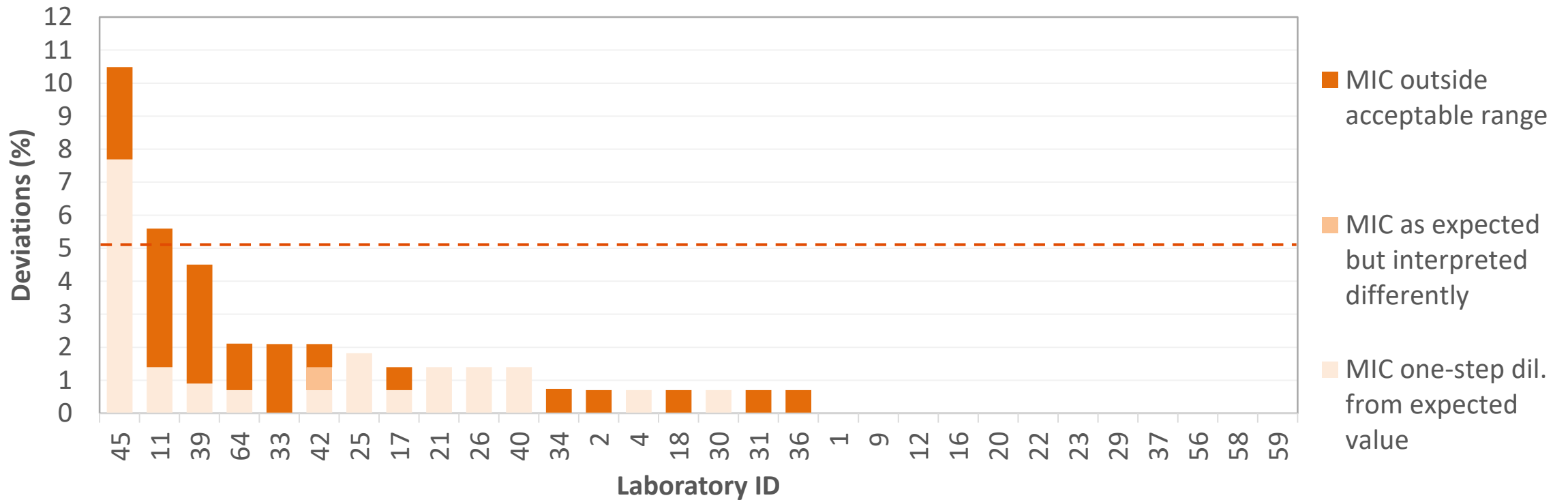
Strain/antimicrobial combinations for which there were > 25% deviations were removed from this graph
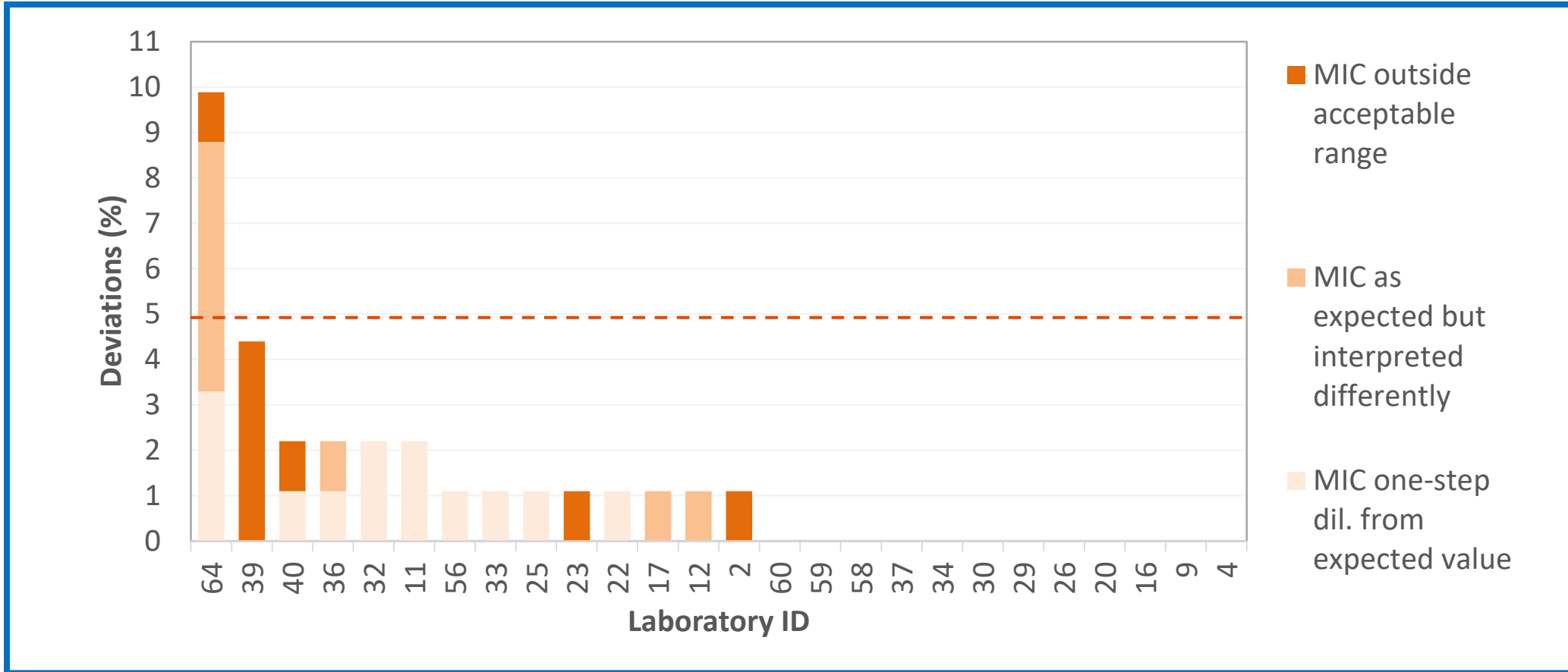
Escherichia coli – you're awesome!

Staphylococcus aureus – good but just a little more effort

Enterococcus sp. – really good, keep going

Detection of antimicrobial resistance phenotypes of particular public health relevance: ESBL/AmpC/carbapenemases

	Strain code	EC-14.1	EC-14.2	EC-14.3	EC-14.4	EC-14.5	EC-14.6	EC-14.7	EC-14.8
	Expected results (based on panel 2 phenotype)	Suscept.	AmpC	AmpC	Suscept.	ESBL	Carbapenemase	ESBL	Carbapenemase
Obtained results	ESBL			1/33 (3.1%)		31/33 (94%)		33/33 (100%)	
	AmpC		32/33 (96.9%)	30/33 (90.9%)					
	ESBL + AmpC		1/33 (3.1%)	2/33 (6%)		2/33 (6%)			
	Carbapenemase						32/33 (96.9%)		33/33 (100%)
	Other						1/33 (3.1%)		
	Susceptible	33/33 (100%)			33/33 (100%)				
	Genetic background	no beta-lactam resistance gene detected	*ampC* promoter (C-42T);	*ampC* promoter (C-42T); *bla*$_{SHV-2}$ (99.8%)	no beta-lactam resistance gene detected	*bla*$_{CTX-M-15}$	*bla*$_{VIM-1}$; *bla*$_{CMY-13}$; *bla*$_{SHV-5}$	*bla*$_{CTX-M-1}$	*bla*$_{OXA-244}$; *bla*$_{CTX-M-14}$

Detection of antimicrobial resistance phenotypes of particular public health relevance: **ESBL/AmpC/carbapenemases**

Strain code	EC-14.1	EC-14.2	EC-14.3	EC-14.4	EC-14.5	EC-14.6	EC-14.7	EC-14.8
Expected results (based on panel 2 phenotype)	Suscept.	AmpC	AmpC	Suscept.	ESBL	Carbapenemase	ESBL	Carbapenemase
Obtained results — ESBL			1/33 (3.1%)		31/33 (94%)			
AmpC		32/33 (96.9%)	30/33 (90.9%)					
ESBL + AmpC		1/33 (3.1%)	2/33 (6%)		2/33 (6%)			
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Other						1/33 (3.1%)		
Susceptible								
Genetic background	no beta-lactam resistance gene detected	*ampC* promoter (C-42T);	*ampC* promoter (C-42T); bla_{SHV-2} (99.8%)	no beta-lactam resistance gene detected	$bla_{CTX-M-15}$	bla_{VIM-1}; bla_{CMY-13}; bla_{SHV-5}		

The phenotype was correct – maybe misunderstanding of EFSA classification

Not based on phenotype but OK if we keep the genetic background in mind

It was correctly interpreted based on the obtained phenotype as FOX was close to ECOFF

Meropenem resistance was not detected but still the Lab recognized that the strain was fishy

Detection of antimicrobial resistance phenotypes of particular public health relevance: **MRSA**

Strain	Phenotype (cefoxitin)	*mec* gene	Correct identification
ST-14.1	MRSA	*mecC*	97%
ST-14.2	MRSA	*mecA*	100%
ST-14.3	MSSA	negative	100%
ST-14.4	MSSA	negative	100%
ST-14.5	MRSA	*mecA*	100%
ST-14.6	MRSA	*mecA*	100%
ST-14.7	MRSA	*mecA*	100%
ST-14.8	MRSA	*mecA*	100%

In three cases, phenotypic test results deviated from expected but the Labs still correctly identified MRSA/MSSA (using genotypic methods and/or latex agglutination methods)

EQAS at a glance: the future is bright!
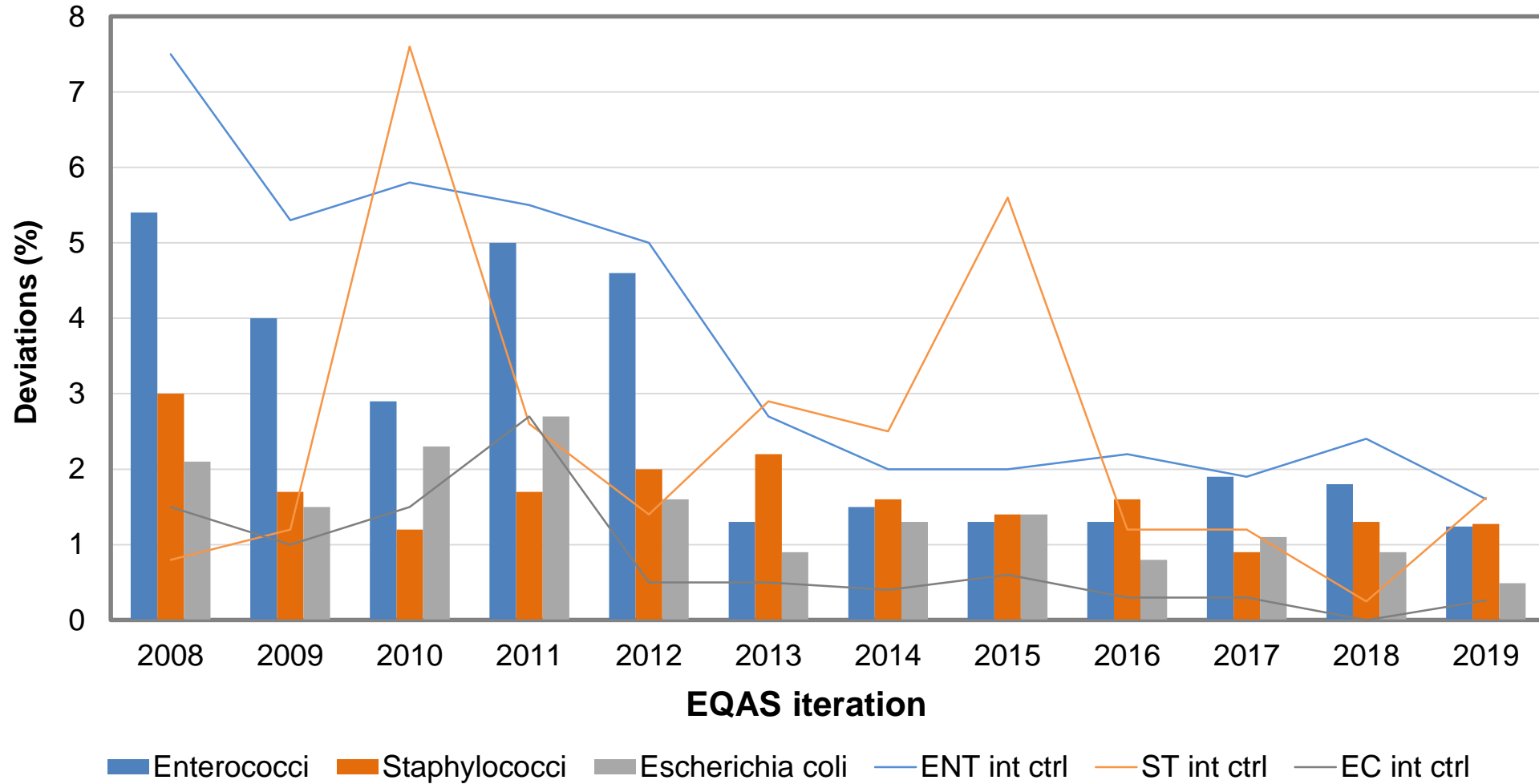
Conclusions

- Overall, excellent performance and no outliers (Lab with > 5% deviations) when correcting for deviations due to limitations in reproducibility of the MIC method

- Room for improvement regarding interpretation of MIC values: cases in which a value is correctly obtained but erroneously interpreted can be easily overcome. Issue of ECOFFs changing over time – how to address it at national and EU level? IMPORTANT LINK:

  https://www.eucast.org/mic_and_zone_distributions_and_ecoffs/new_and_revised_ecoffs/

- ESBL/AmpC categorization: minor issues mainly related to definitions. Molecular methods highlights genetic background that was overlooked by using phenotype only

- MRSA detection: usefulness of molecular and/or latex agglutination methods to complement phenotypic test results

- Alert regarding carbapenemase detection: as they are infrequent at present (luckily), they might be difficult to detect. Re-test any isolate that looks suspicious to you

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Thank you for your attention!

Questions?