

Overall outcomes of the EURL-AR EQAS 2020 for *Escherichia coli*, *Salmonella* and *Campylobacter* sp.

Susanne Karlsdose Pedersen
EU Reference Laboratory for Antimicrobial Resistance
Technical University of Denmark

What is evaluated in this EQAS

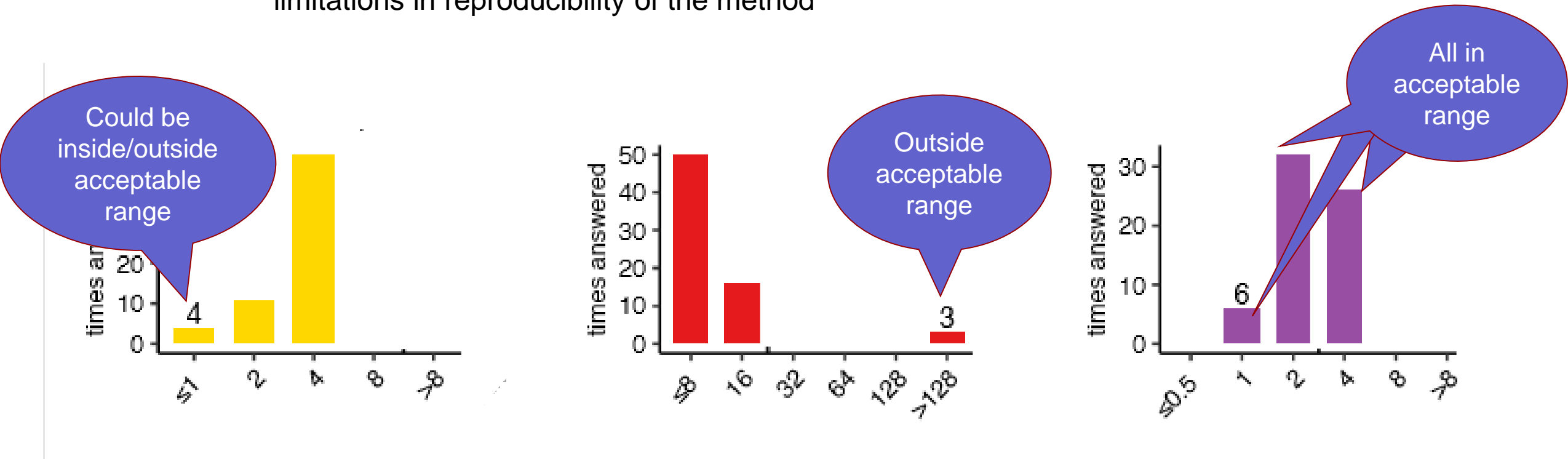
- AST of 8 *E. coli*, 8 *Salmonella* and 8 *Campylobacter* test strains
 - MIC determination
 - Interpretation according to EUCAST ECOFFs
- Detection of resistance phenotypes of particular public health relevance
 - ESBL/AmpC/carbapenemase production in *E. coli* and *Salmonella*
- Species identification of *Campylobacter*
- Test of ATCC strains for QC

Evaluation = presence/absence of deviations

Deviations: let's set the record straight

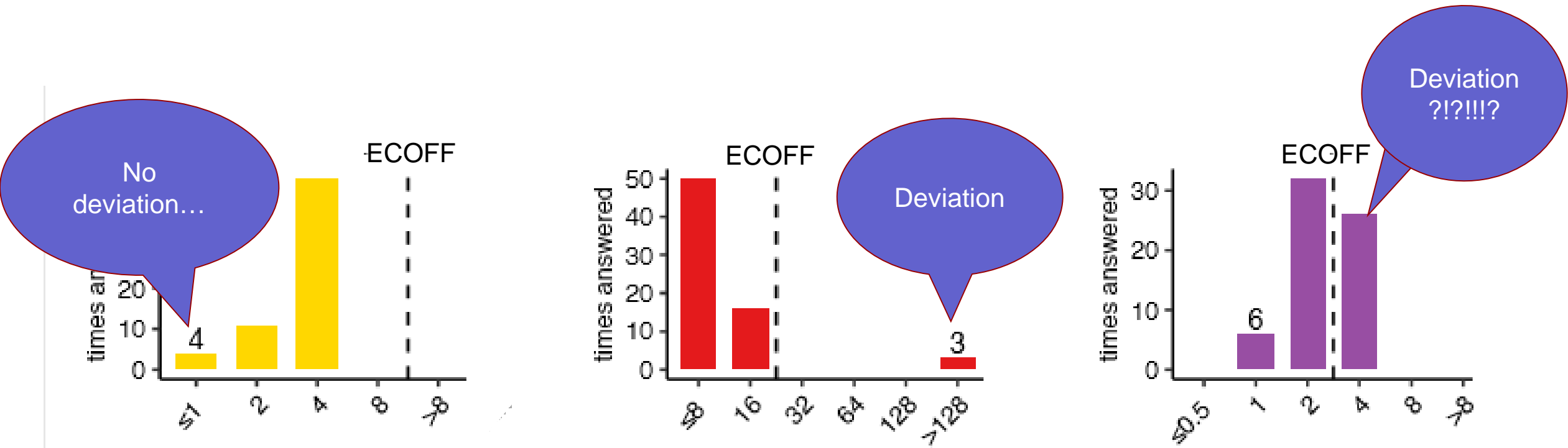
A **deviation** is due to obtained interpretation different from the expected interpretation.
But...

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

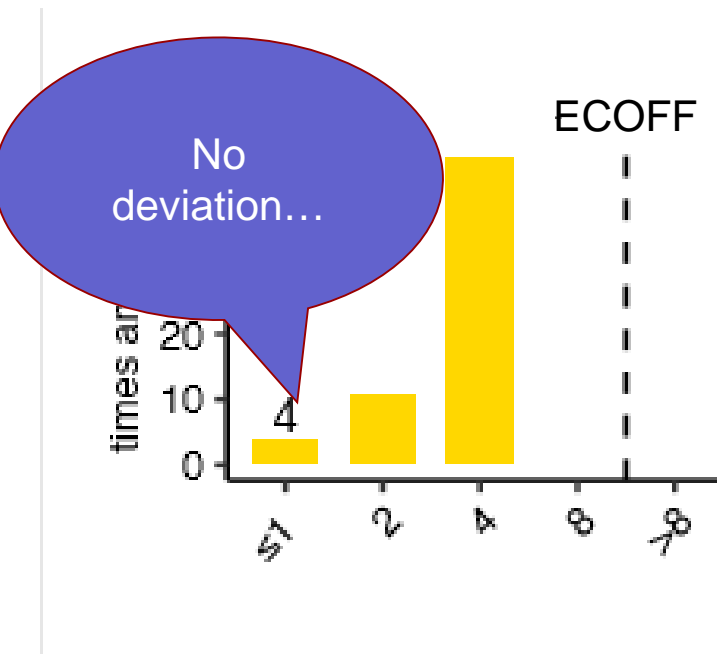


Deviations: let's set the record straight

If the 'right' MIC is close to the ECOFF then different interpretations will be obtained – and one of them will be scored as a deviation - for MIC values which are otherwise in the acceptable range



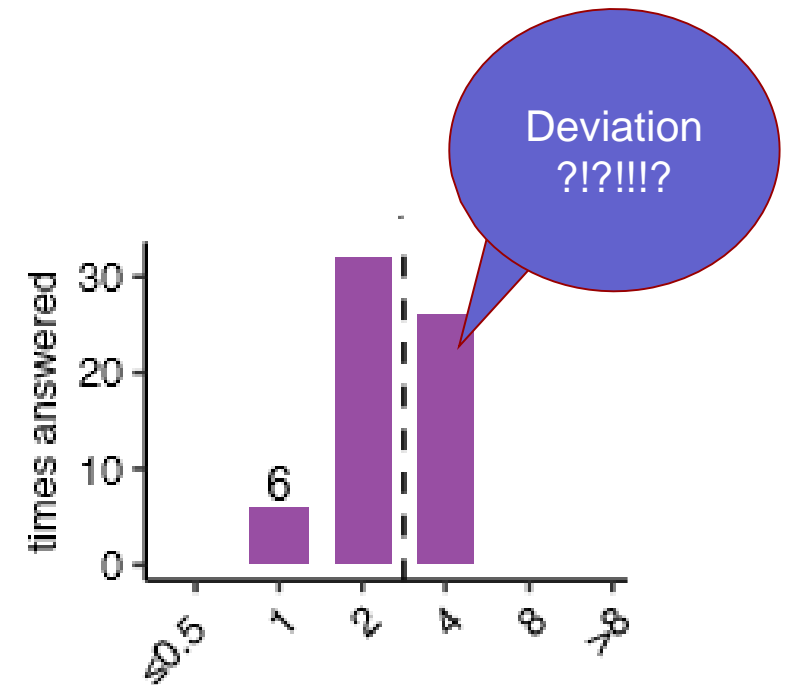
Sometimes, a MIC obtained in the acceptable range is **interpreted erroneously**... distraction issue, easy to overcome or use of different interpretive criteria...



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

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**"



Drug/bug combinations omitted from analysis (deviation level > 25%)

For *E. coli*:

- **EC-15.1/F/C** (one-fold dilution issues)
- **EC-15.2/CHL** (one-fold dilution issues)
- **EC-15.2/FOT (panel 1)** (one-fold dilution issues)
 - For panel 2, 100% of submitted interpretations corresponded with the expected!
- **EC15.7/SMX** (18/30 obtained an unexpected result as resistant)

For *Salmonella*:

- **S-15.6/TAZ** (panel 1 and 2) (one-fold dilution issues)

For *Campylobacter*: None omitted

Drug/bug combinations omitted from analysis (deviation level > 25%)

For *E. coli*:

- **EC-15.1/F/C** (one-fold dilution issues)
- **EC-15.2/CHL** (one-fold dilution issues)
- **EC-15.2/FOT (panel 1)** (one-fold dilution issues)
 - For panel 2, 100% of submitted interpretations corresponded with the expected!
- **EC15.7/SMX** (18/30 obtained an unexpected result as resistant)

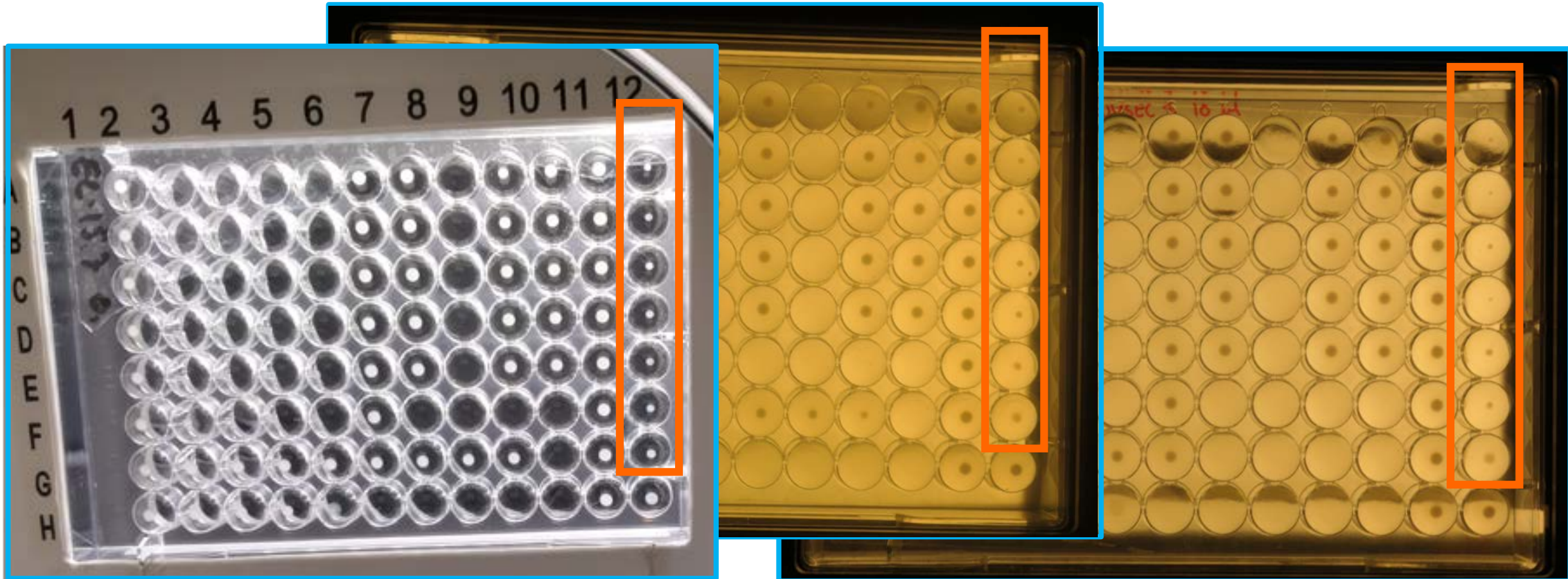
For *Salmonella*:

- **S-15.6/TAZ** (panel 1 and 2) (one-fold dilution issues)

For *Campylobacter*: None omitted

EC15.7/SMX – results omitted

- 18/30 obtained an unexpected result as resistant



EC15.7/SMX – genotypic background

Genomic analysis related to sulfonamide resistance:
sul2, detected (one base is missing!)

Folate pathway antagonist									
Resistance gene	Identity	Alignment Length/Gene Length	Position in reference	Contig or Depth	Position in contig	Phenotype	PMID	Accession no.	
sul2	99.88	816/816	1..817	61.78	None..None	sulfamethoxazole	22888274	HQ840942	

```

sul2, ID: 99.88 %, Alignment Length/Gene Length: 816/816, Coverage: 100.0, Positions in reference: 1..817, Contig name: NA, Position: NA

Resistance gene seq: ATGAATAAATCGCTCATCATTTTCGGCATCGTCAACATAACCTCGGACAGTTTCTCCGAT
Hit in genome:      ATGAATAAATCGCTCATCATTTTCGGCATCGTCAACATAACCTCGGACAGTTTCTCCGAT

Resistance gene seq: GGAGGCCGGTATCTGGCGCCAGACGCAGCCATTGCGCAGGCGCGTAAGCTGATGGCCGAG
Hit in genome:      GGAGGCCGGTATCTGGCGCCAGACGCAGCCATTGCGCAGGCGCGTAAGCTGATGGCCGAG

Resistance gene seq: GGGGCAGATGTGATCGACCTCGGTCCGGCATCCAGCAACCCCGACGCCGCGCCTGTTTCG
Hit in genome:      GGGGCAGATGTGATCGACCTCGGTCCGGCATCCAGCAACCCCGACGCCGCGCCTGTTTCG

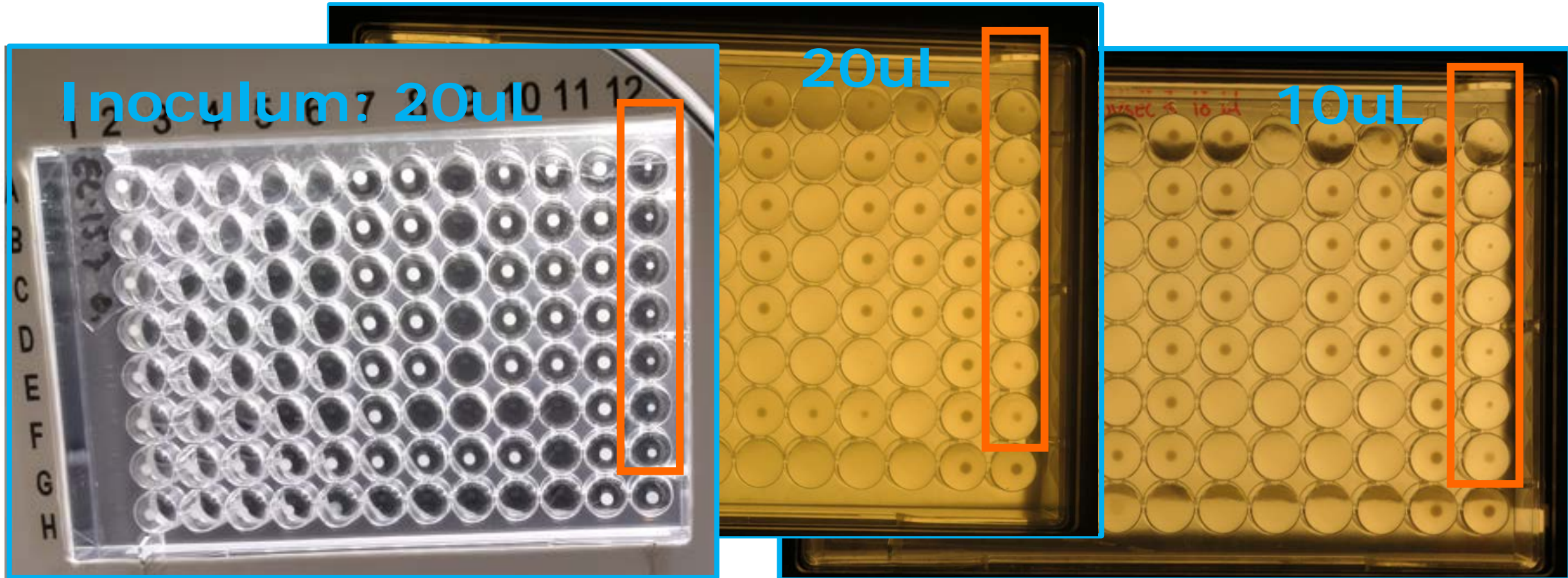
Resistance gene seq: TCCGACACAGAAATCGAGCGTATCGCGCCGGTCTGGACGCGCTCAAGGCAGATGGCATT
Hit in genome:      TCCGACACAGAAATCGAGCGTATCGCGCCGGTCTGGACGCGCTCAAGGCAGATGGCATT

Resistance gene seq: CCCGTCTCGCTCGACAGTTATCAACCCGCGACGCAAGCCTATGCCTTGTGCGGTGGTGTG
Hit in genome:      CCCGTCTCGCTCGACAGTTATCAACCCGCGACGCAAGCCTATGCCTTGTGCGGTGGTGTG
  
```

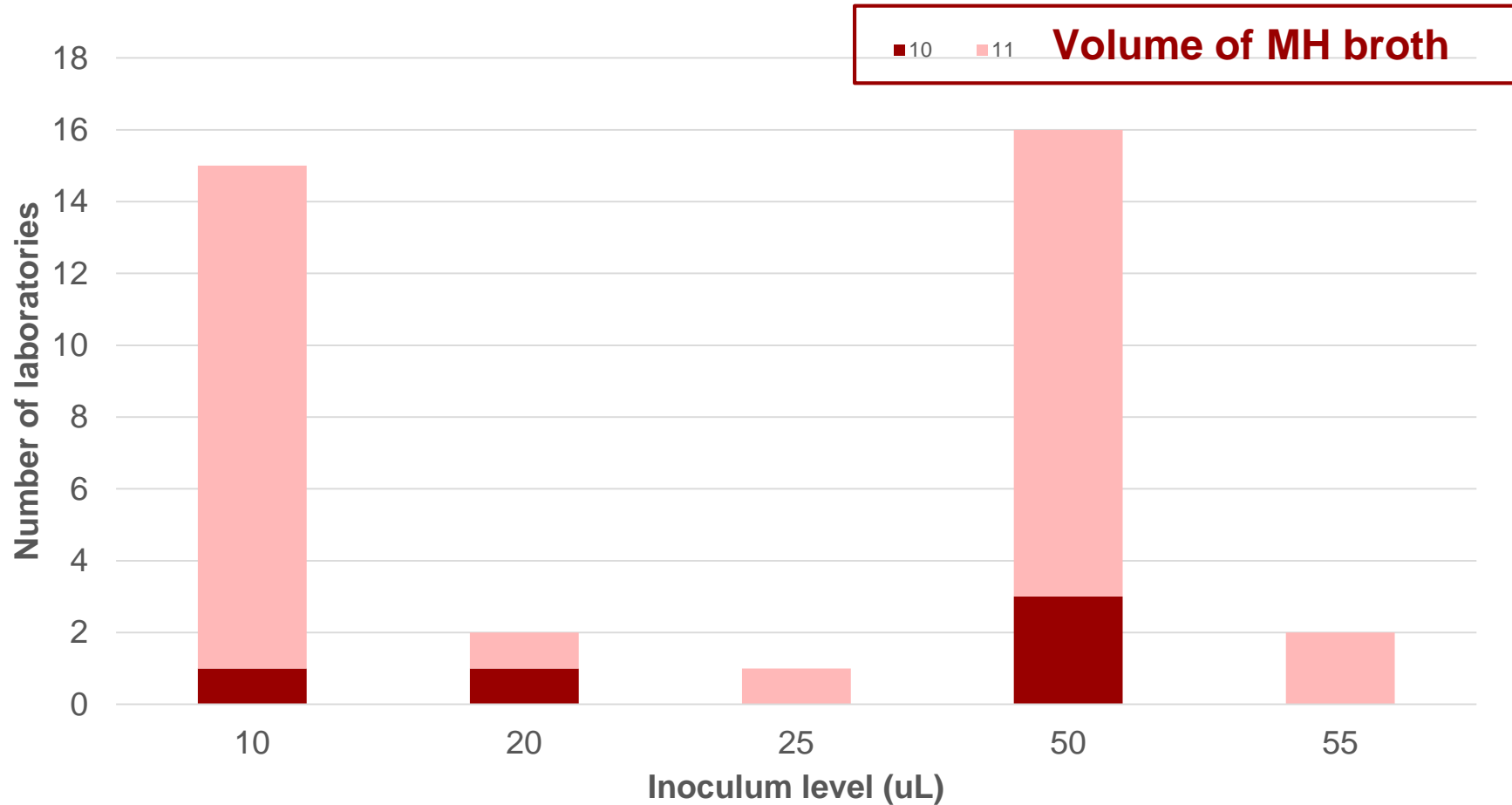
=> May be the reason why the strain was observed to express different phenotypes when tested in different laboratories, as the gene could potentially be expressed to some extent, or not.

EC15.7/SMX – results omitted

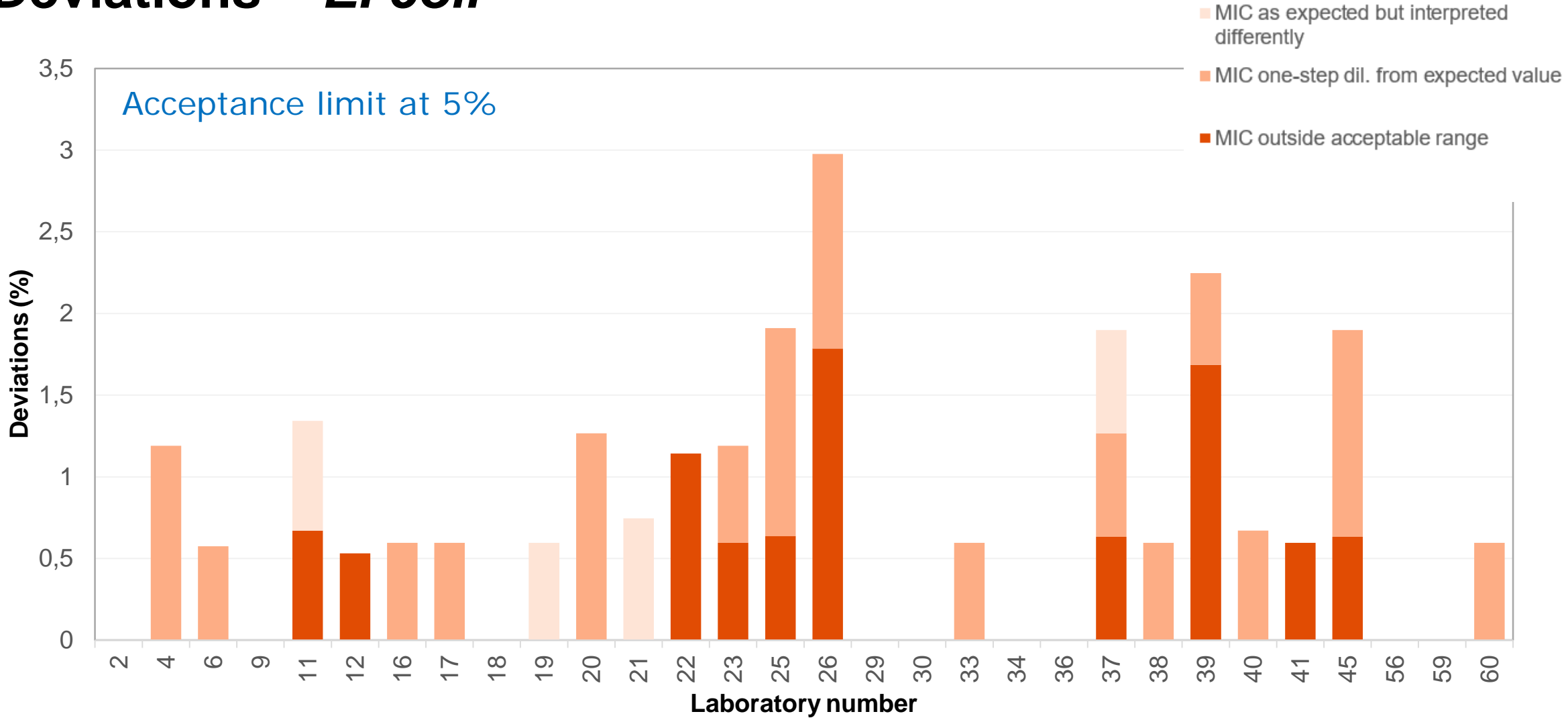
- 18/30 obtained an unexpected result as resistant



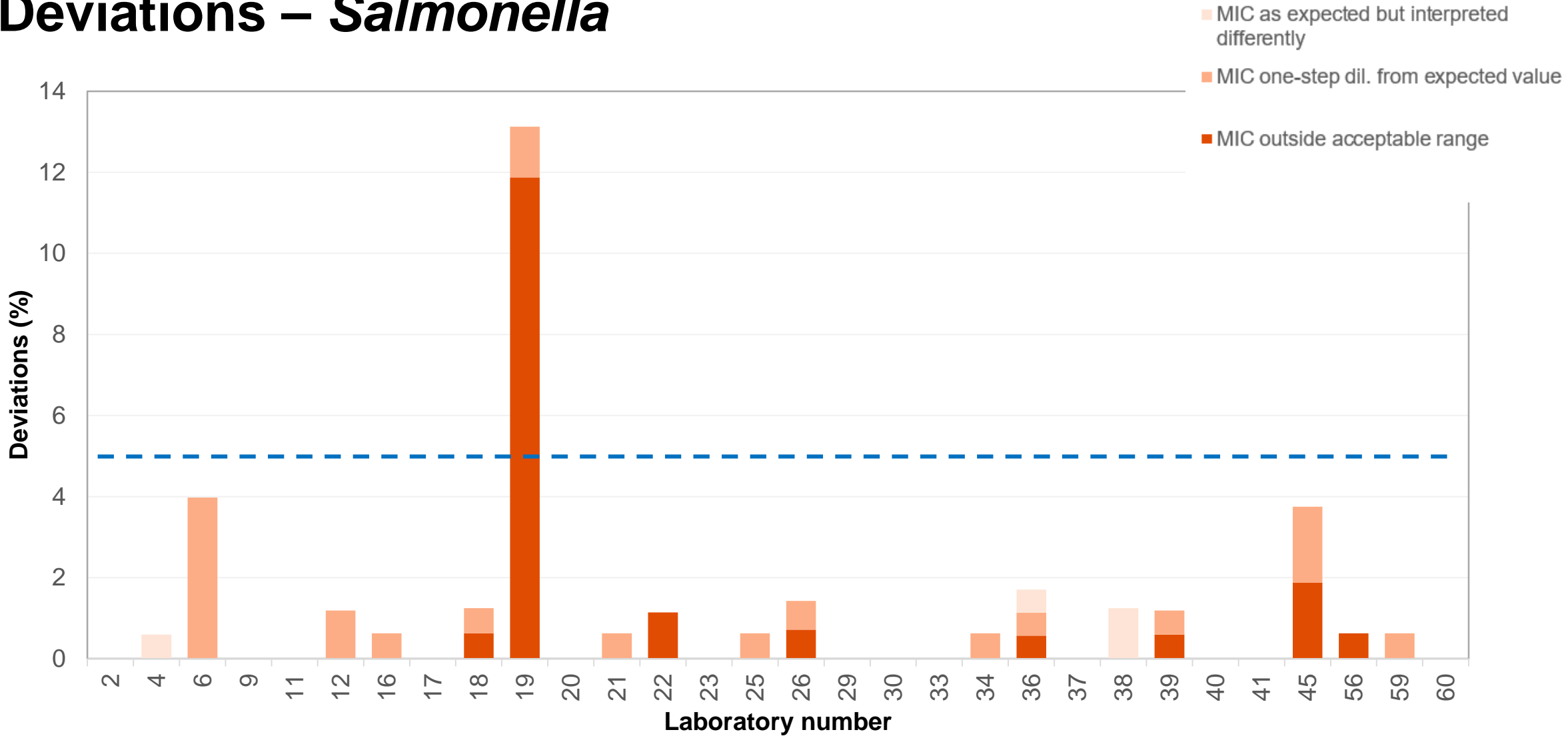
Inoculum level – *Salmonella*



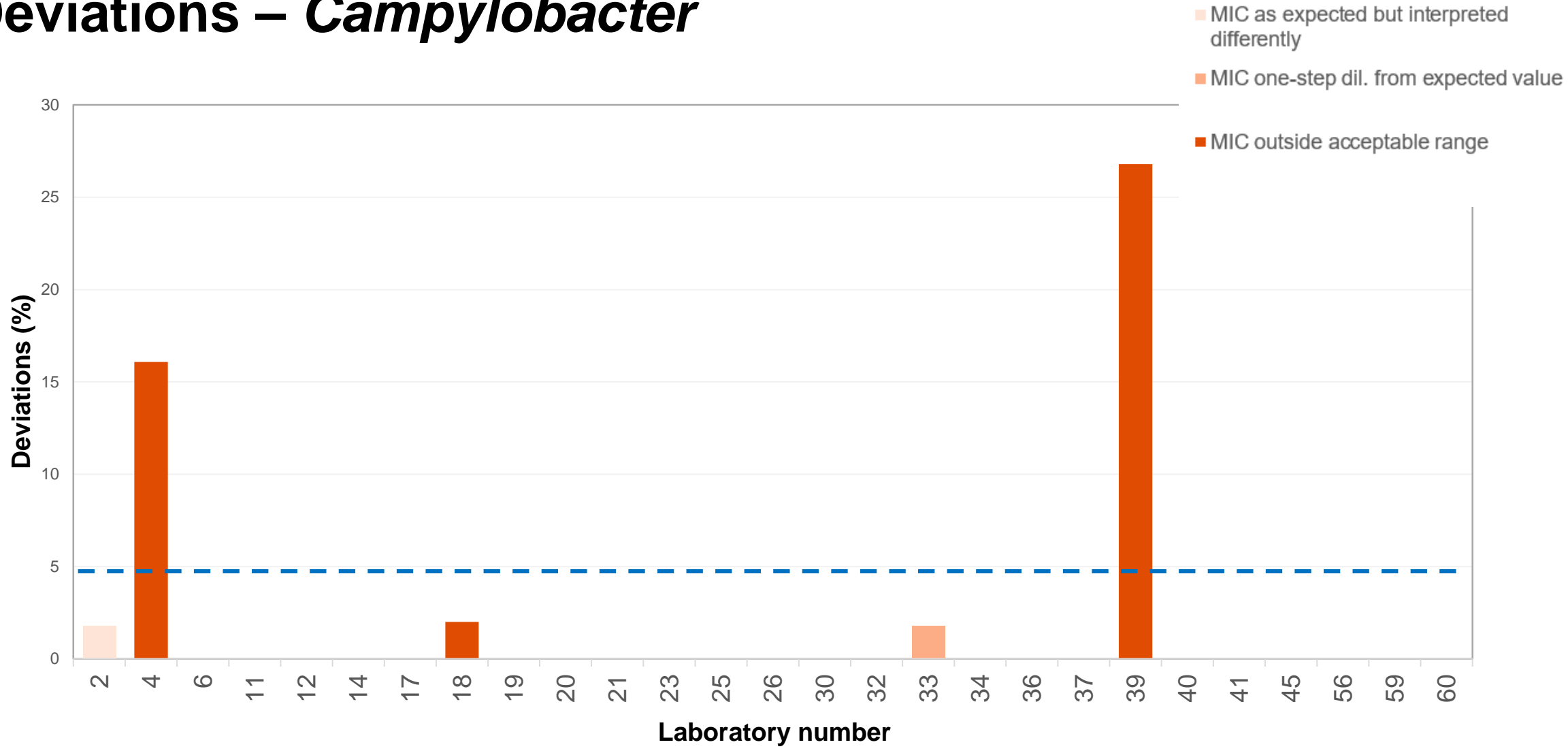
Deviations – *E. coli*



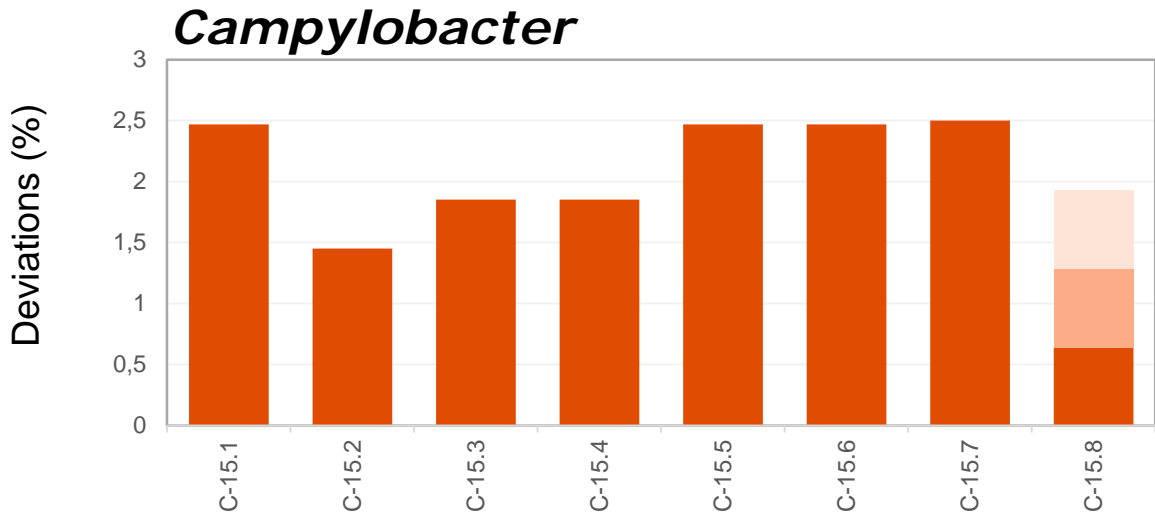
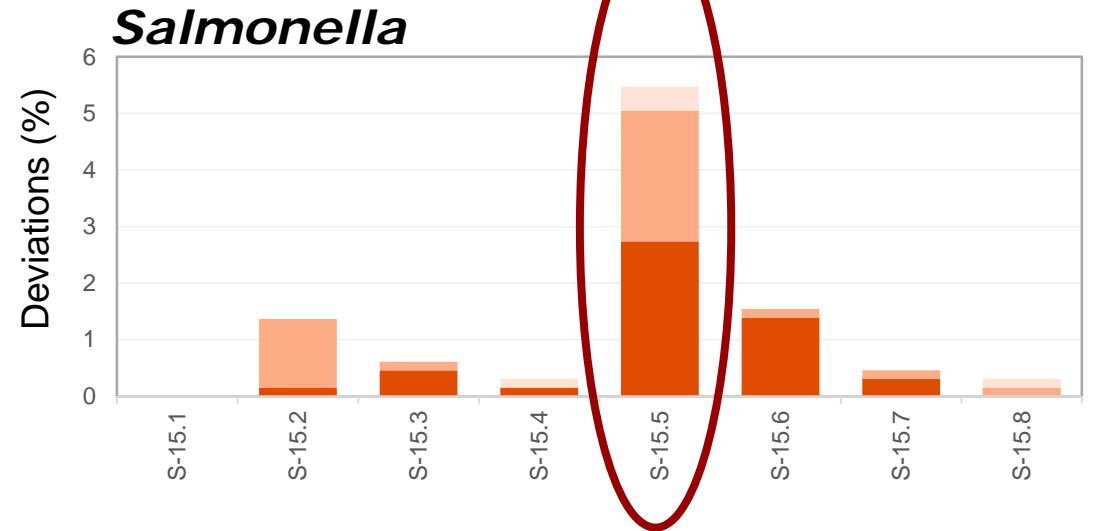
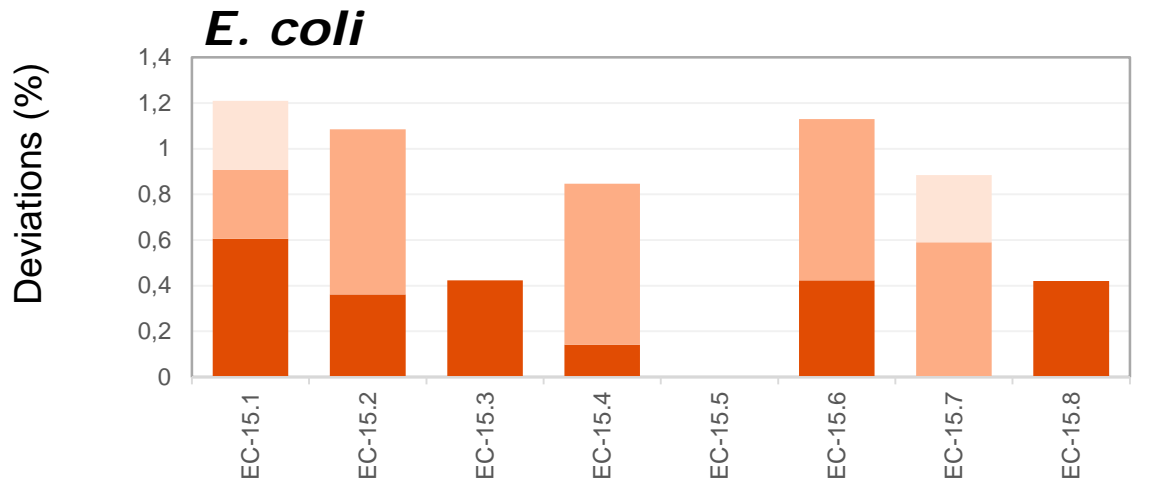
Deviations – *Salmonella*



Deviations – *Campylobacter*



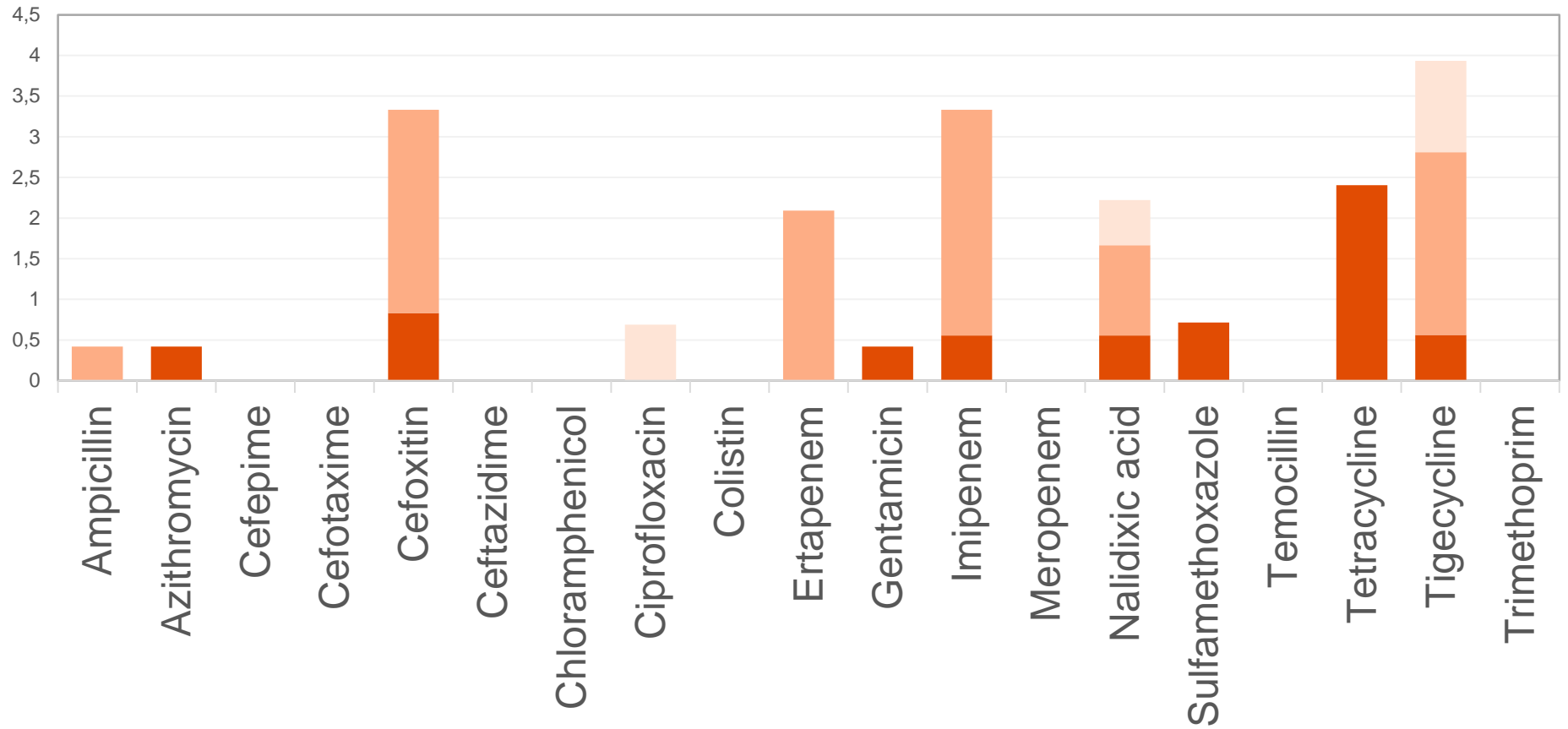
Deviations – by strain



- MIC as expected but interpreted differently
- MIC one-step dil. from expected value
- MIC outside acceptable range

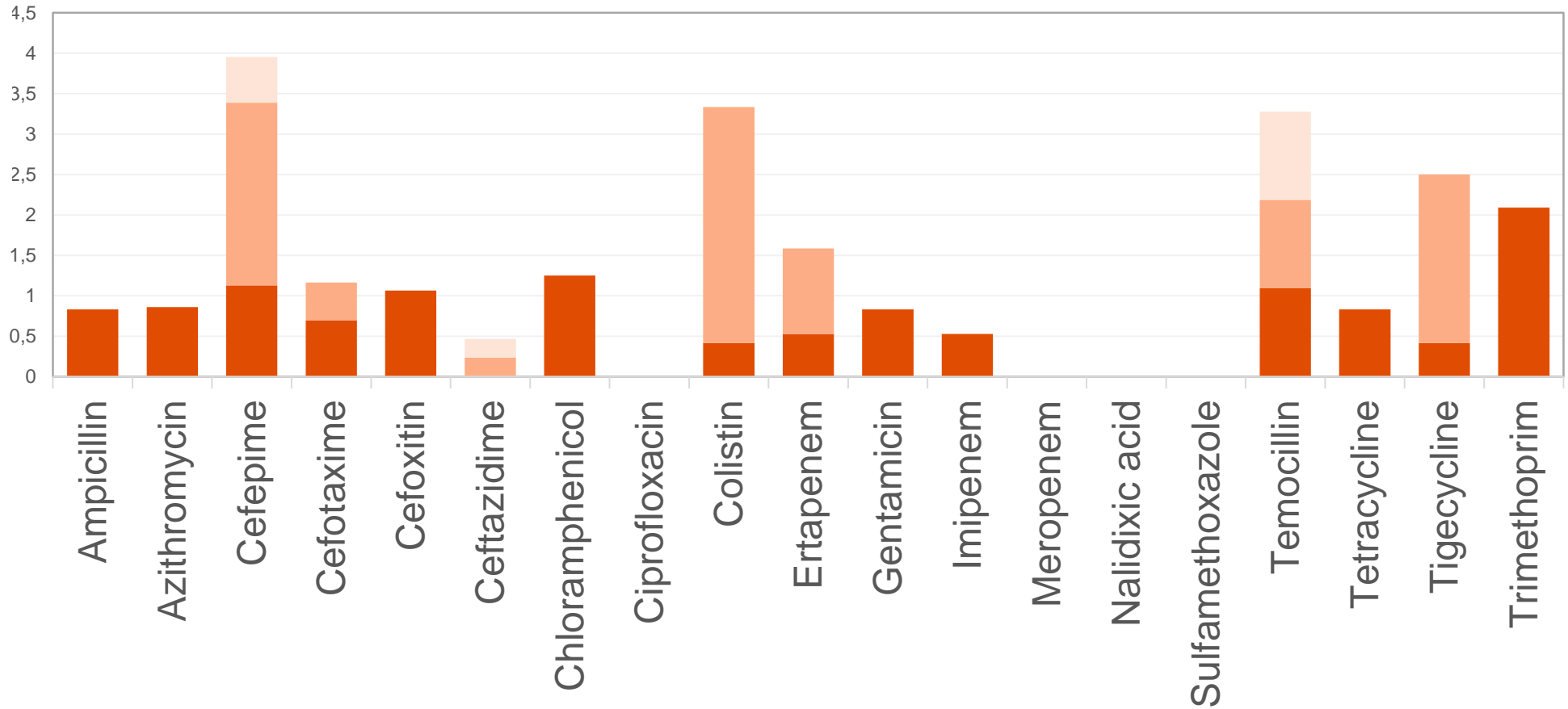
Deviations by antimicrobials – *E. coli*

- MIC as expected but interpreted differently
- MIC one-step dil. from expected value
- MIC outside acceptable range



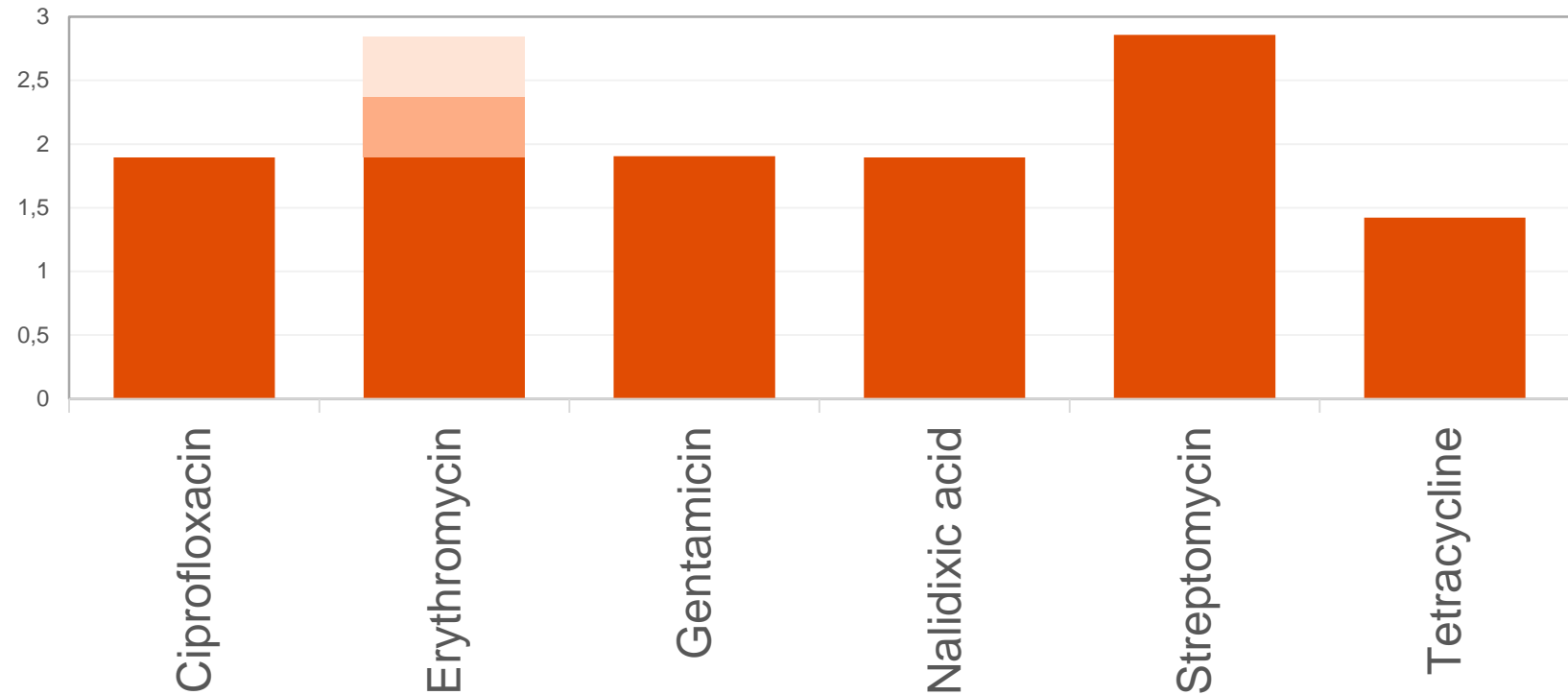
Deviations by antimicrobials – *Salmonella*

- MIC as expected but interpreted differently
- MIC one-step dil. from expected value
- MIC outside acceptable range



Deviations by antimicrobials – *Campylobacter*

- MIC as expected but interpreted differently
- MIC one-step dil. from expected value
- MIC outside acceptable range



Detection of antimicrobial resistance phenotypes of particular public health relevance: ESBL/AmpC/carbapenemases – *E. coli*

The phenotype was correct – maybe misunderstanding of EFSA classification

	EC-15.4	EC-15.5	EC-15.6	EC-15.7	EC-15.8			
(based on panel 2 phenotype)	Carbapenemase	AmpC/Other	ESBL	ESBL	Susceptible	ESBL+AmpC	Carbapenemase	Susceptible
ESBL	1/29 (3.4%)	-	30/30 (100%)	30/30 (100%)	-	-	-	-
AmpC	-	9/21 (42.9%)	-	-	-	1/30 (3.3%)	-	-
ESBL + AmpC	1/29 (3.4%)	-	-	-	-	28/30 (93.3%)	-	-
Carbapenemase	26/29 (89.7%)	-	-	-	-	-	30/30 (100%)	-
Other	1/29 (3.4%)	10/21 (47.6%)	-	-	-	1/30 (3.3%)	-	1/3 (33.3%)
Susceptible	-	2/21 (9.5%)	-	-	2/2 (100%)	-	-	2/3 (66.6%)
Genetic background	<i>bla</i> _{CTX-M-14}	No ESBL gene or mutation detected	<i>bla</i> _{CTX-M-1}	<i>bla</i> _{CTX-M-1}	No ESBL gene or mutation detected	<i>bla</i> _{CMY-2} <i>bla</i> _{CTX-M-27}	<i>bla</i> _{NDM-5} <i>bla</i> _{SHV-12}	No ESBL gene or mutation detected

Consistent with the laboratory's obtained phenotypic results

Obtained results

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

Strain code	S-15.1	S-15.2	S-15.3	S-15.4	S-15.5	S-15.6	S-15.7	S-15.8	
Expected results (based on panel 2 phenotype)	Susceptible	ESBL	AmpC	ESBL	Other	ESBL	Carbapenemase	ESBL	
Observed results	ESBL	-	30/30 (100%)	-	30/30 (100%)	1/6 (16.7%)	29/29 (100%)	-	30/30 (100%)
	AmpC	-	-	29/30 (96.7%)	-	2/6 (33.3%)	Unusual profile...		-
	ESBL + AmpC	-	-	1/30 (3.3%)	-	-	-	-	-
	Other	-	-	-	-	-	30/30 (100%)	-	-
	Susceptible	2/2 (100%)	-	-	-	1/6 (16.7%)	-	-	-
	Susceptible	-	-	-	-	2/6 (33.3%)	-	-	-
Genetic background	No ESBL gene or mutation detected	<i>bla</i> _{CTX-M-3} <i>bla</i> _{OXA-1}	<i>bla</i> _{CMY-2}	<i>bla</i> _{CTX-M-14b}	No ESBL gene or mutation detected	<i>bla</i> _{CTX-M-14}	<i>bla</i> _{NDM-1} <i>bla</i> _{CMY-4} <i>bla</i> _{CMY-16}	<i>bla</i> _{CTX-M-9}	

The phenotype was correct – maybe misunderstanding of EFSA classification

S-15.5 – an unusual AST profile

Strain code		S-15.5
Expected results (based on panel 2 phenotype)		Other
Obtained results	ESBL	1/6 (16.7%)
	AmpC	2/6 (33.3%)
	ESBL + AmpC	-
	Carbapenemase	-
	Other	1/6 (16.7%)
	Susceptible	2/6 (33.3%)
Genetic background		No ESBL gene or mutation detected

- Data from **S15.5/FEP/FOT/FOX/TRM** (panel 2)) resulted in $\geq 25\%$ deviations – *results not omitted from analysis and report*
- Panel 2 data was submitted by seven laboratories.
- Five of these – and one additional laboratory – submitted an ESBL category
- **FOX > 32**, though no ESBL gene or mutation detected

S15.5 – AST results from panel 2

AST	FOT Obtained panel 1	FOT Obtained panel 2
Expected	0.5 / S	0.5 / S
#4	0.5 / S	0.5 / S
#6	1 / R	1 / R
#11	0.25 / S	-
#12	0.5 / S	0.5 / S
#19	4 / R	16 / R
#22	0.25 / S	0.25 / S
#36	0.5 / S	0.5 / S
#39	0.5 / S	0.5 / S

S-15.5 – an unusual AST profile

2. AmpC-Phenotype

- FOT or TAZ > 1 mg/L AND
- MERO ≤ 0.12 mg/L AND
- FOX > 8 mg/L AND
- No SYN FOT/CLV nor TAZ/CLV
- (Not excluded presence of ESBLs)

Due to switch of strains?

Consistent with the laboratory's obtained phenotypic results

ESBL Interpretation	Panel 2 required	ESBL category	FOT >1	TAZ >1	FOX > 8	Synergy	MERO >0.12
Expected	no	Other	no	no	YES	no	no
#4	YES	AmpC*	no	no	YES	no	no
#6	YES	AmpC*	no	no	YES	no	no
#11	no	Susc	no	no	Panel 2 not tested	Panel 2 not tested	no
#12	no	-	no	no	YES	no	no
#19	YES	ESBL	YES	YES	No (MIC=4)	YES	no
#22	YES	Susc	no	no	No (MIC=8)	no	no
#36	no	-	no	no	YES	no	no
#39	YES	Other	no	no	YES	no	no

* Incorrect, though understandable categorization

Conclusions

- Overall, for *E. coli*, excellent performance and no outliers (i.e. no lab with > 5% deviations)
- For *Salmonella*, excellent performance.
Though, one outlier was observed (switch of strains)
- For *Campylobacter*, excellent performance.
Though, two outliers were observed (switch of strains/contamination)
- ESBL/AmpC categorization: minor issues mainly related to definitions

Thank you for your attention

Any questions?