DTU Food
National Food Institute

European Union Reference Laboratory Antimicrobial Resistance

EURL-AR EQAS 2011

Enterococci, staphylococci and *Escherichia coli*

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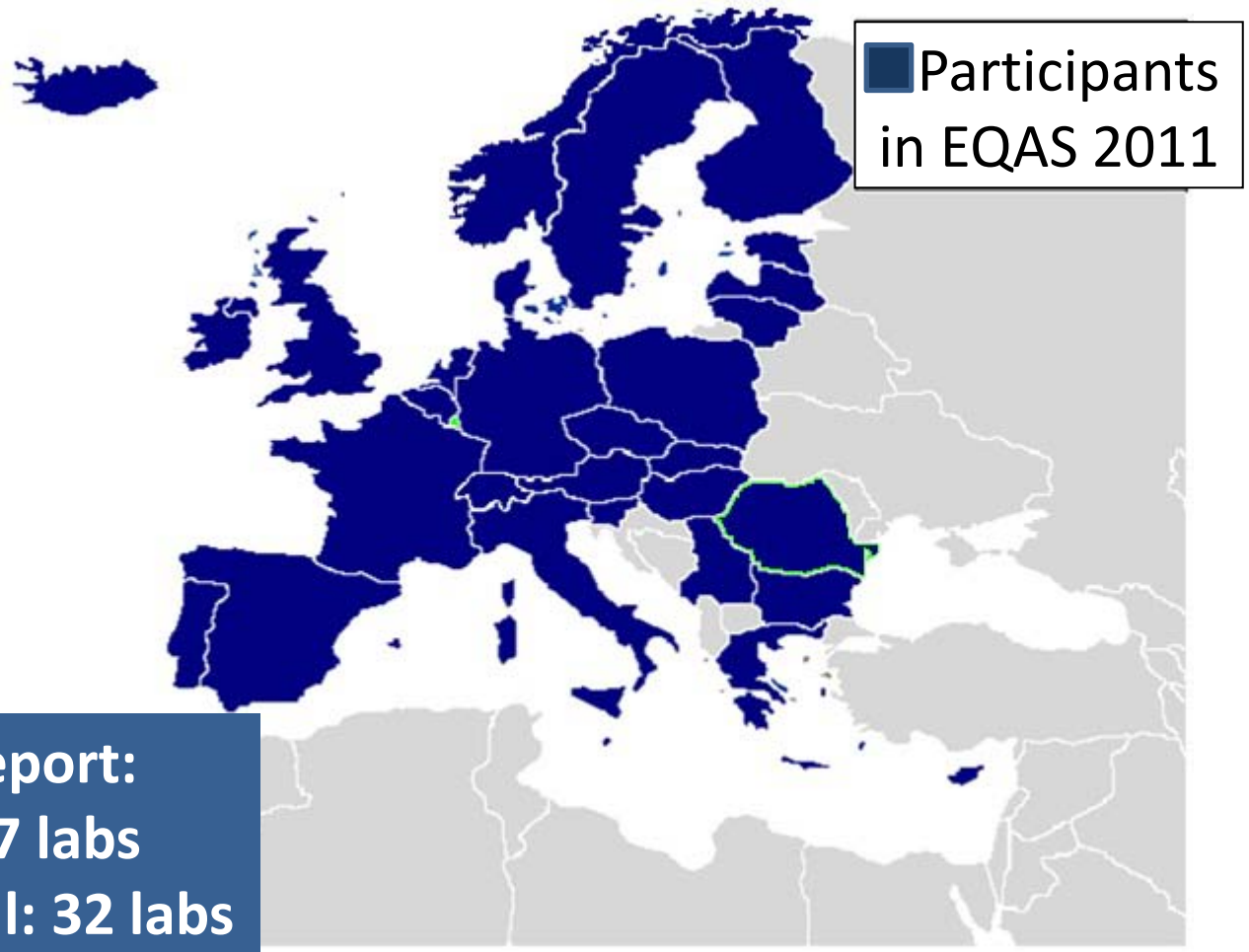
EURL-AR workshop
Lyngby, Denmark - 23 April 2012

Outline

- Methods (participants, strains, antimicrobials, …)
- Objectives of this proficiency test
- General overview of the results
- Overview of results and conclusions for:
  - Enterococci trial
  - Staphylococci trial
  - *Escherichia coli* trial

Participants in the enterococci, staphylococci and *E. coli* EQAS, 2011



Evaluation report:
- **ENT trial: 27 labs**
- **ST & EC trial: 32 labs**

Methods for EQAS 2011

- Eight strains of enterococci, staphylococci and *E. coli* were selected

- New participants were provided with reference strains: *E. faecalis* ATCC 29212, *S. aureus* ATCC 25923 or *S. aureus* ATCC 29213 and *E. coli* ATCC 25922 for quality control purposes

- Antimicrobial susceptibility testing (AST) guidelines were according to CLSI. **Results had to be interpreted to categorise the strains as resistant or susceptible**

Methods for EQAS 2011

- MIC results were interpreted using the EUCAST epidemiological cut-off values (www.eucast.org), as recommended by EFSA and described in the protocol

- Participants using disk diffusion were encouraged to interpret the results according to their routinely applied breakpoints

Antimicrobials recommended

Enterococci trial	Staphylococci trial*	Escherichia coli trial
Ampicillin†	Cefoxitin	Ampicillin†
Chloramphenicol†	Chloramphenicol	Cefotaxime†
Ciprofloxacin	Ciprofloxacin	Cefoxitin
Erythromycin†	Erythromycin	Ceftazidime
Gentamicin†	Florfenicol	Ceftiofur
Linezolid†	Gentamicin	Chloramphenicol†
Streptomycin†	Penicillin	Ciprofloxacin†
Quinu-dalfopristin†	Streptomycin	Florfenicol
Tetracycline†	Sulphonamides	Gentamicin†
Vancomycin†	Tetracycline	Nalidixic acid†
	Trimethoprim	Streptomycin†
		Sulphonamides†
		Tetracycline†

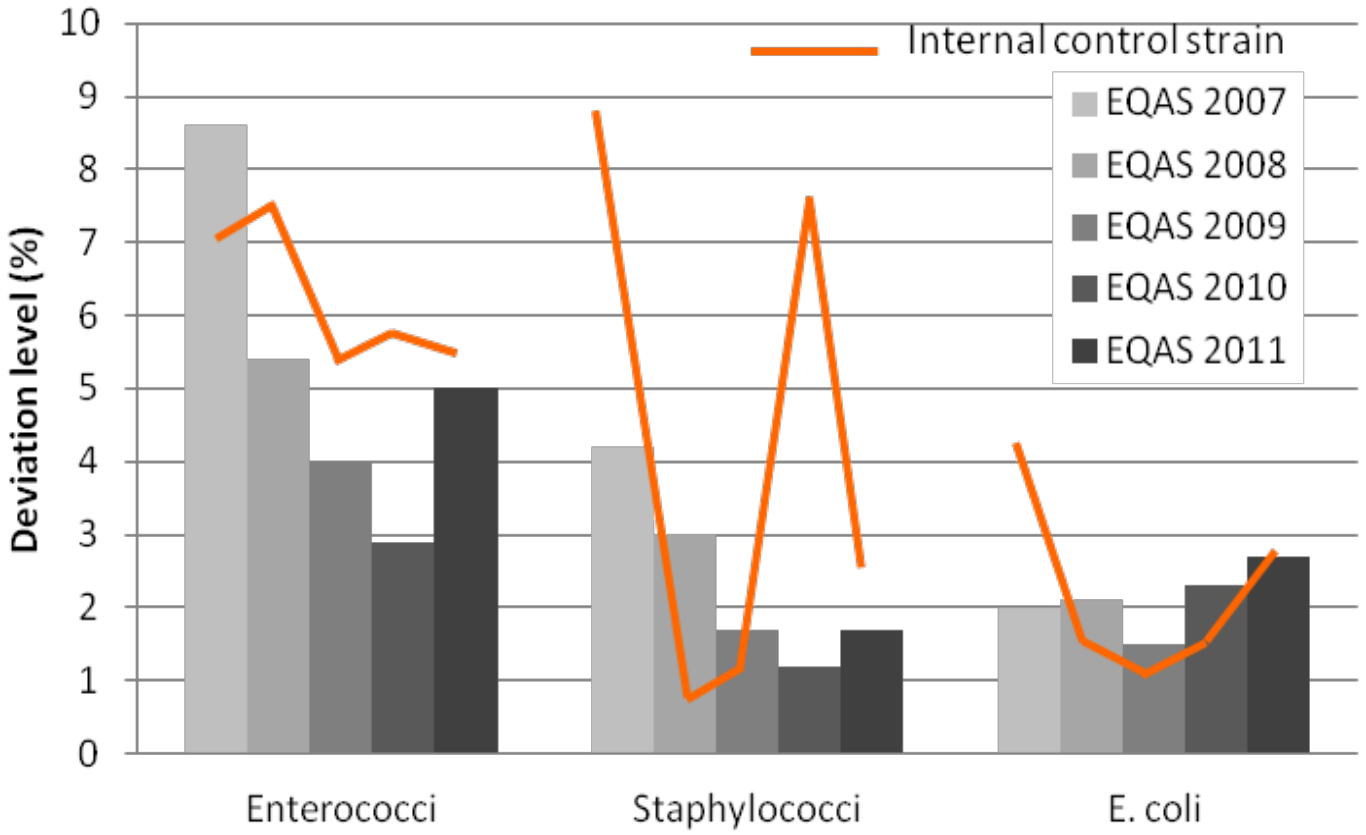
Main objectives of the EURL-AR EQAS

- To assess the quality of AST results in European laboratories
- To support laboratories in performing, evaluating and, if necessary, improving AST
- To improve the comparability of AST results within EU countries
- To harmonise the breakpoints/epidemiological cut-off values for AST results interpretation

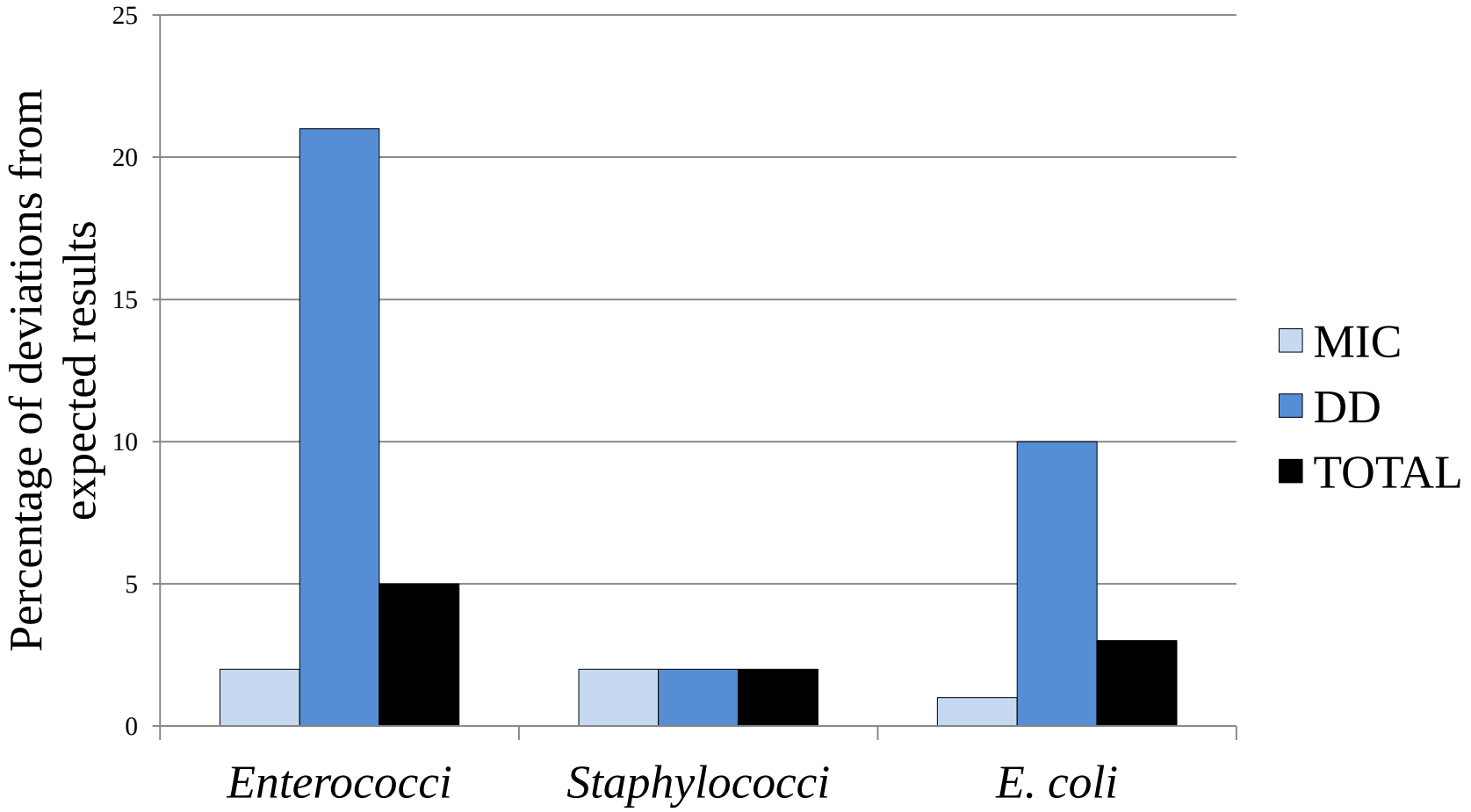
Data analysis

- **Evaluation is based on interpretation of results**
- If less than **75%** of the results for a specific strain/antimicrobial combination are in agreement with the expected interpretation, further analysis is performed to decide to keep or delete these results from the overall evaluation
- Participants obtaining more than **5%** of the results deviating from the expected interpretation will be contacted to identify problems in AST and propose possible solutions

EQAS 2011 compared to previous EQAS iterations



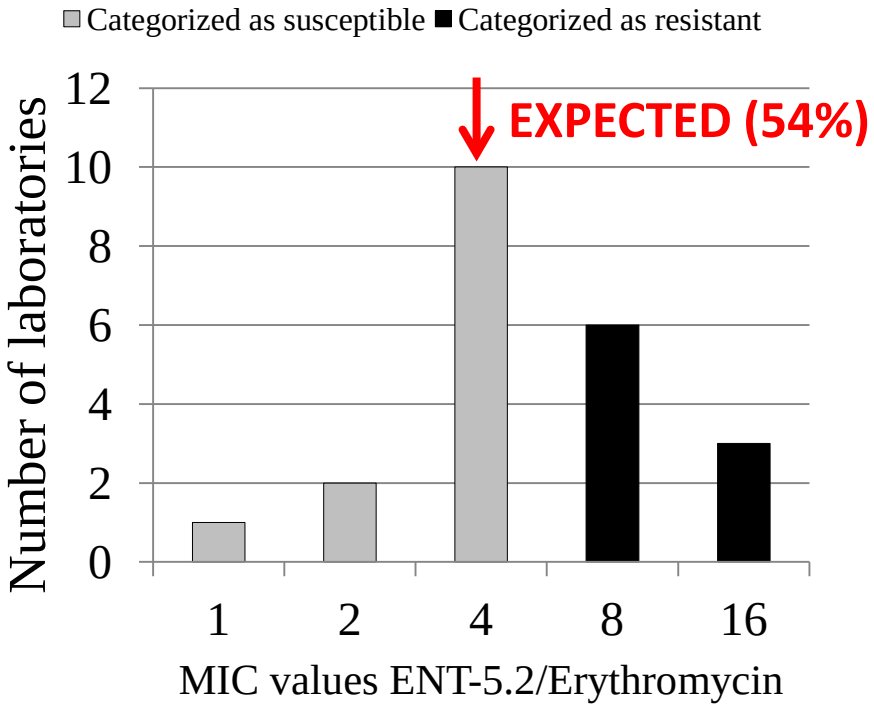
Deviations from expected results by bacterial species and AST method



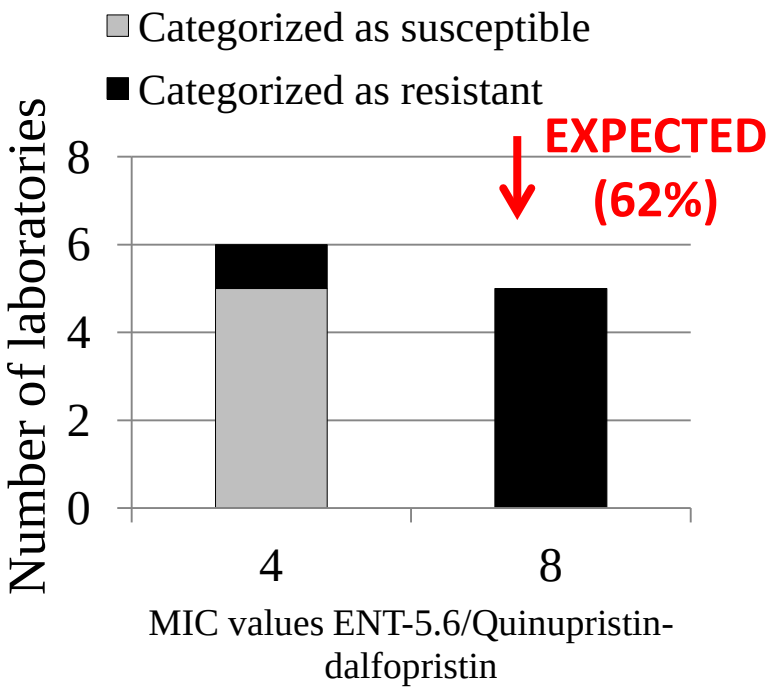
Enterococci trial

Results omitted:

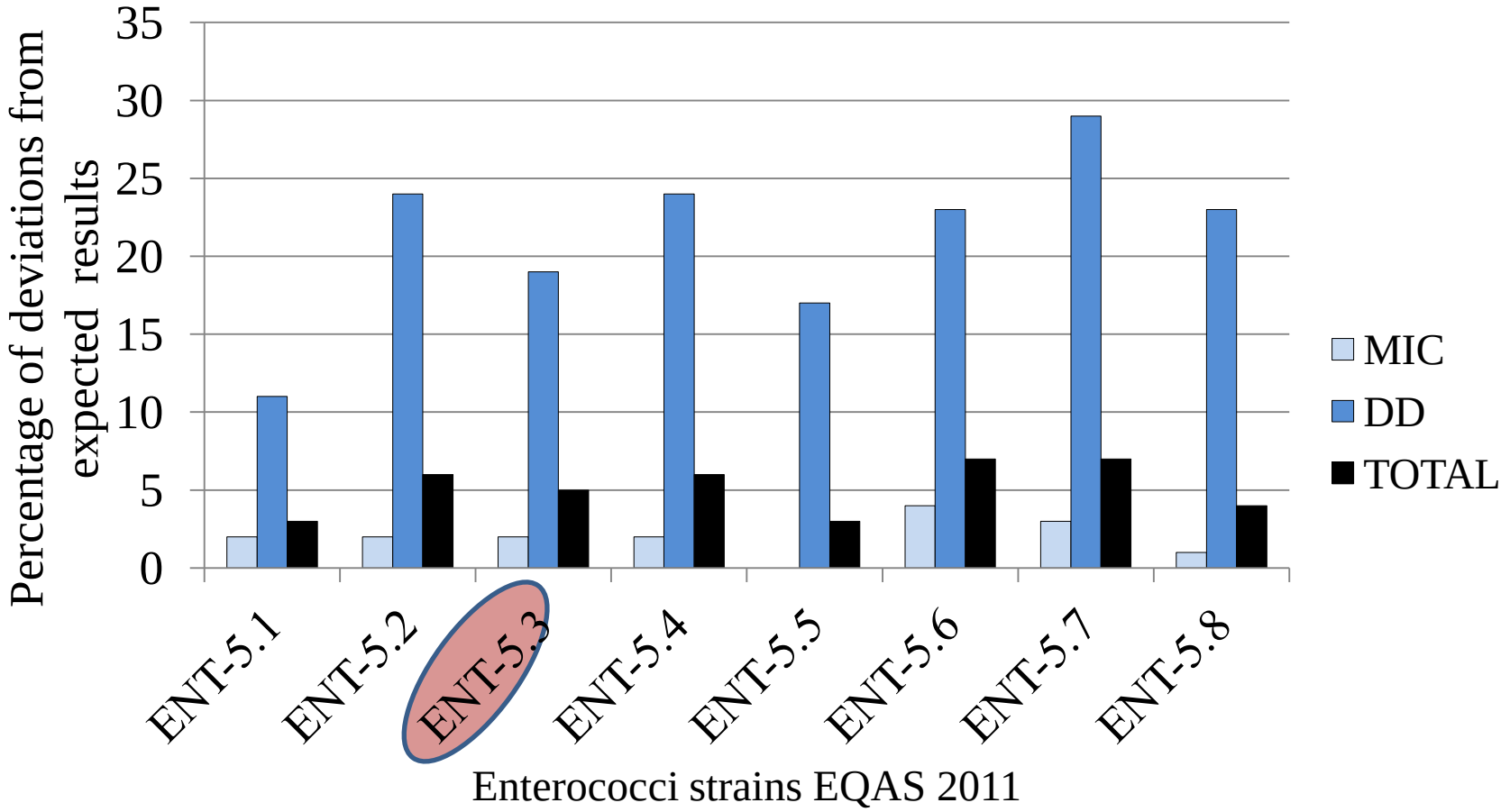
ENT-5.2/ERY



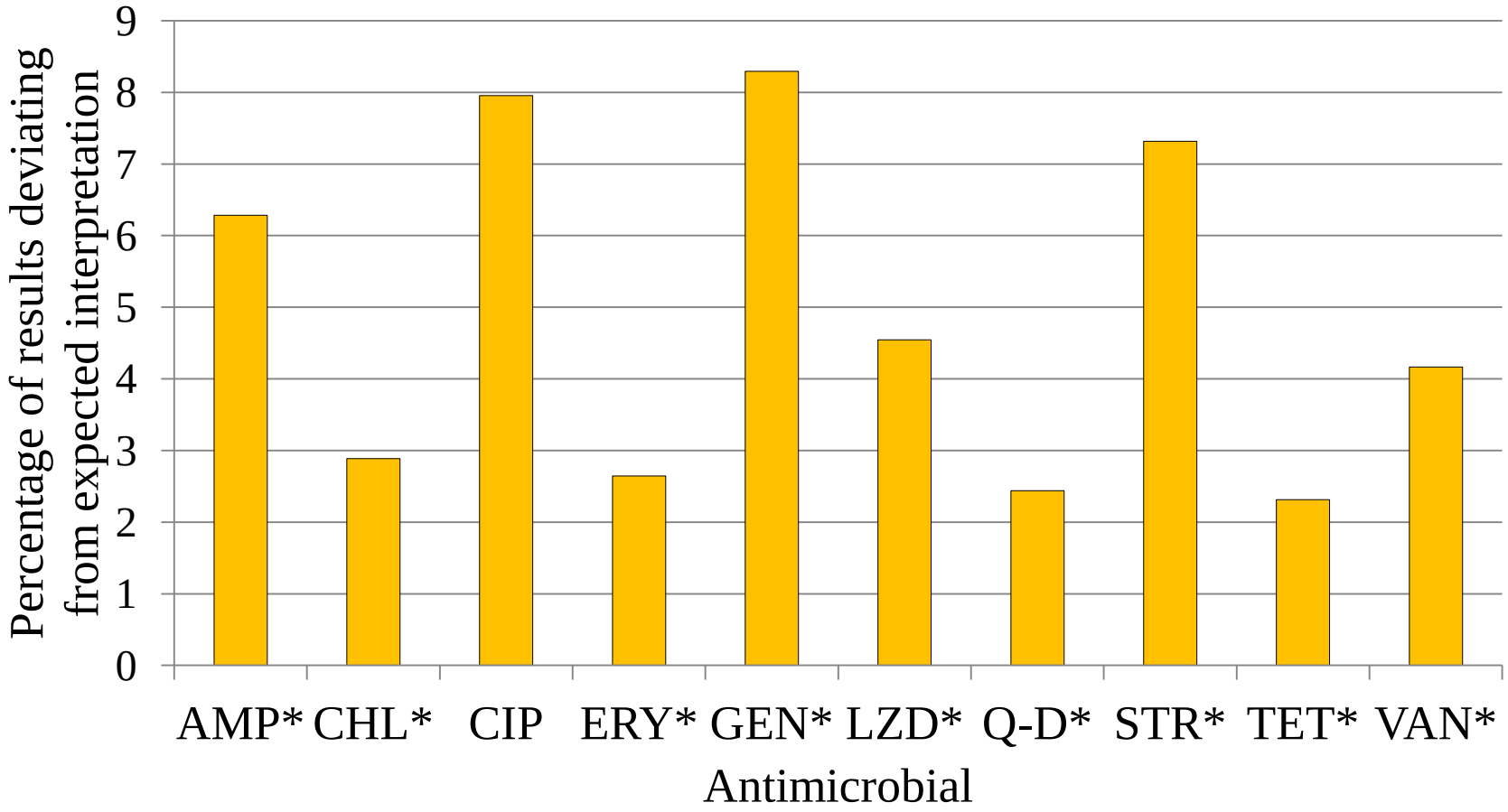
ENT-5.6/Q-D



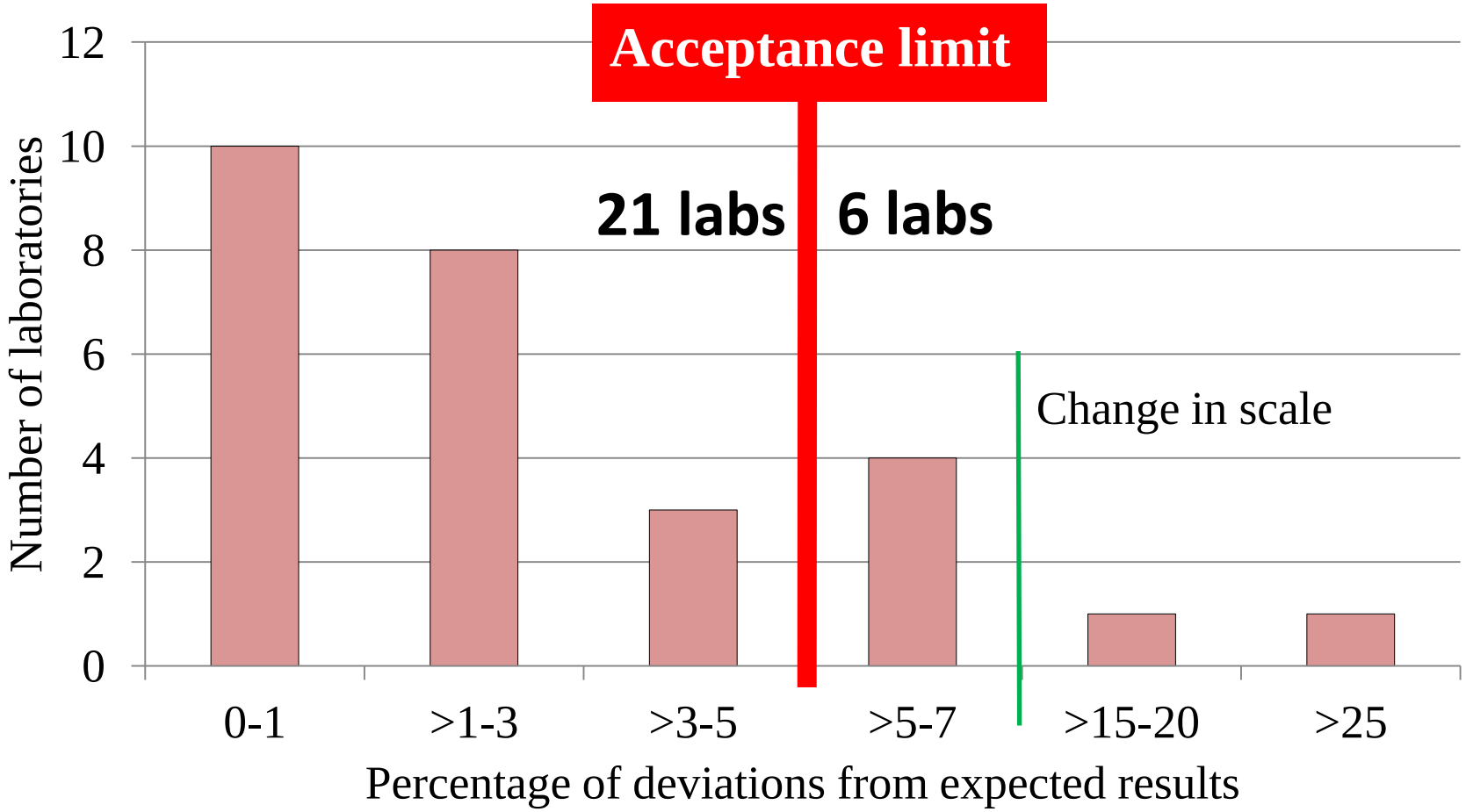
Deviations from expected results by strain tested and AST method used



Deviations from expected results by antimicrobial tested



Overall lab. performance in enterococci trial



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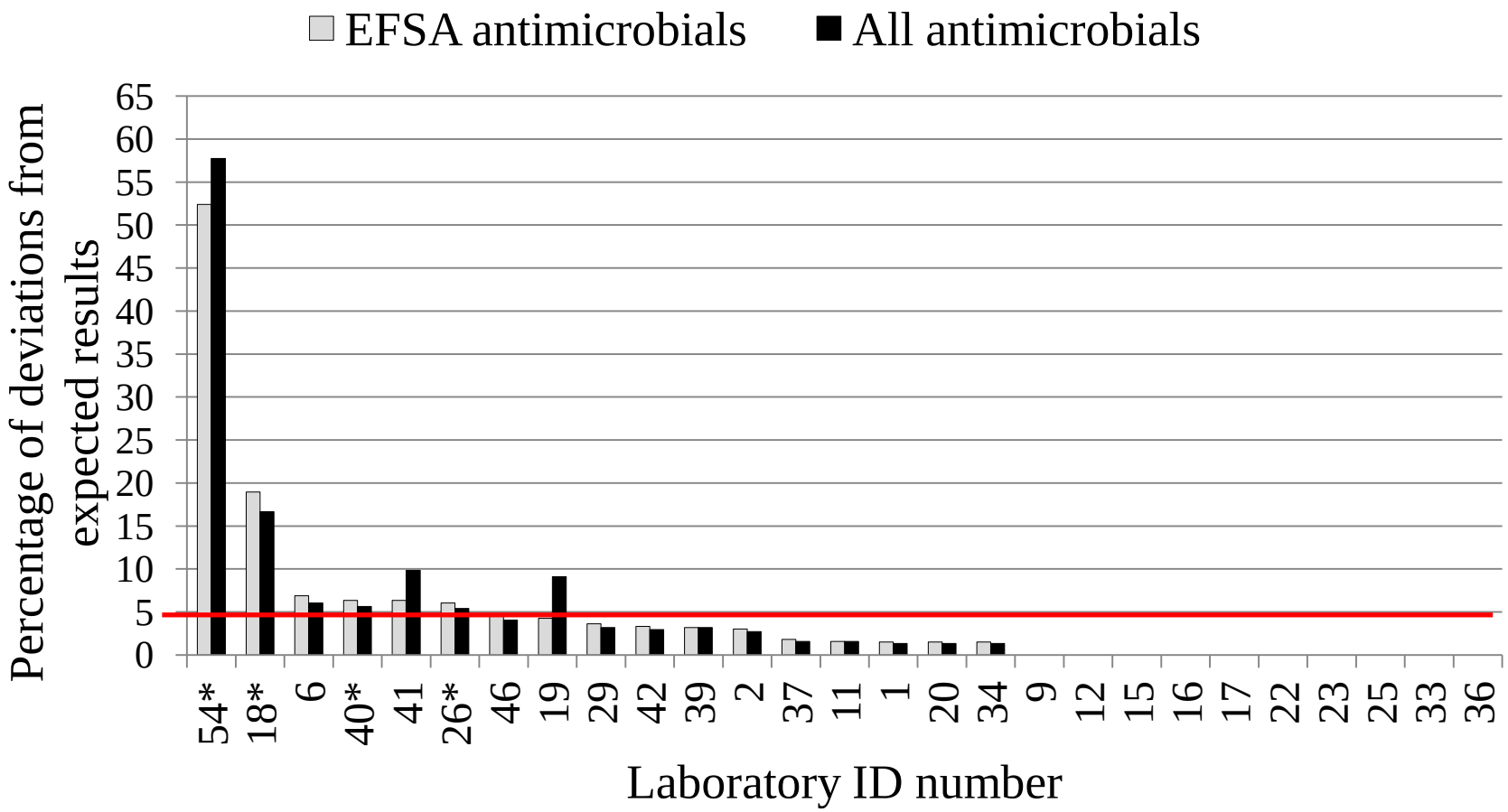


Quality Control
Enterococcus faecalis ATCC 29212

Antimicrobial	QC range (MIC)	Min. value	Max. value	No. of deviations from expected result/ Total no. of tests
Ampicillin	0.5 - 2	1	**4**	**1/19**
Chloramphenicol	1 - 4	4	8	0/20
Ciprofloxacin	0.25 - 2	0.5	1	0/16
Erythromycin	1 - 4	**0.25**	4	**2/21**
Gentamicin	4 - 16	4	≤ 128	0/21
Linezolid	1 - 4	1	2	0/18
Quinu-dalfopristin	2 - 8	2	8	0/10
Streptomycin	n.a.*	32	512	0/20
Tetracycline	8 - 32	**4**	32	**2/21**
Vancomycin	1 - 4	1	4	0/21

*n.a., not applicable

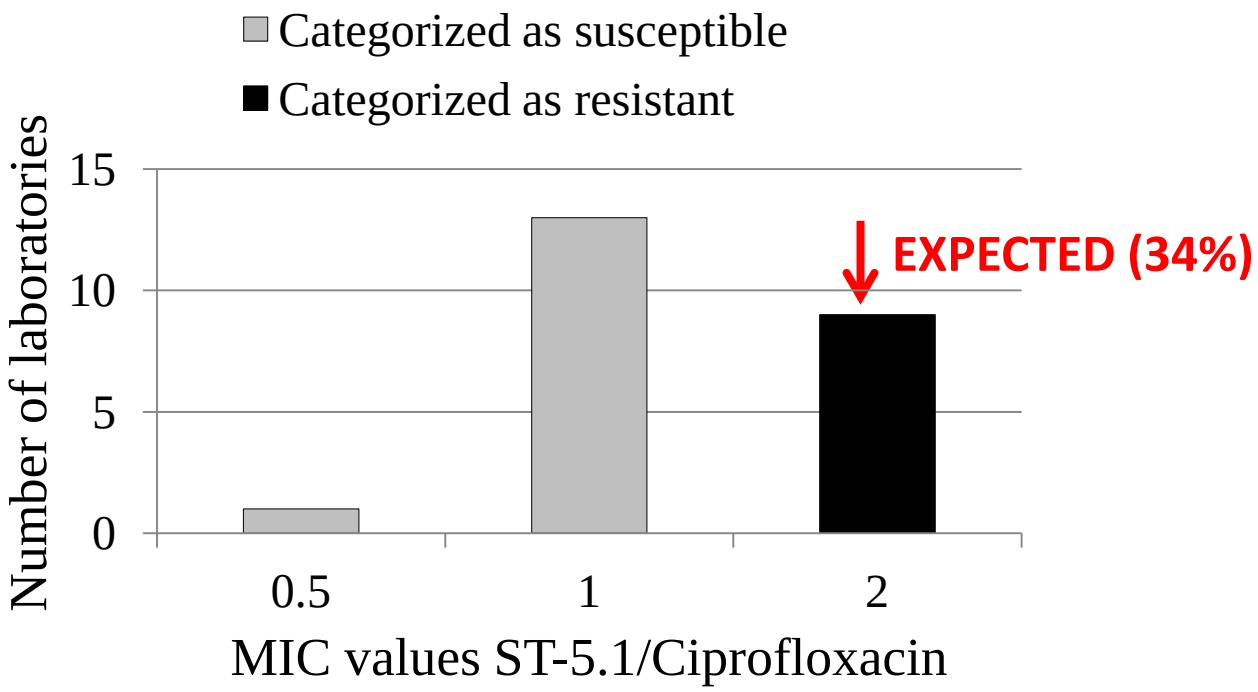
Conclusions (enterococci)

- 27 participants (23: MIC; 4: DD)
- 6 laboratories outside the acceptance limit
- Test of QC strain was quite satisfactory but there is room for improvement

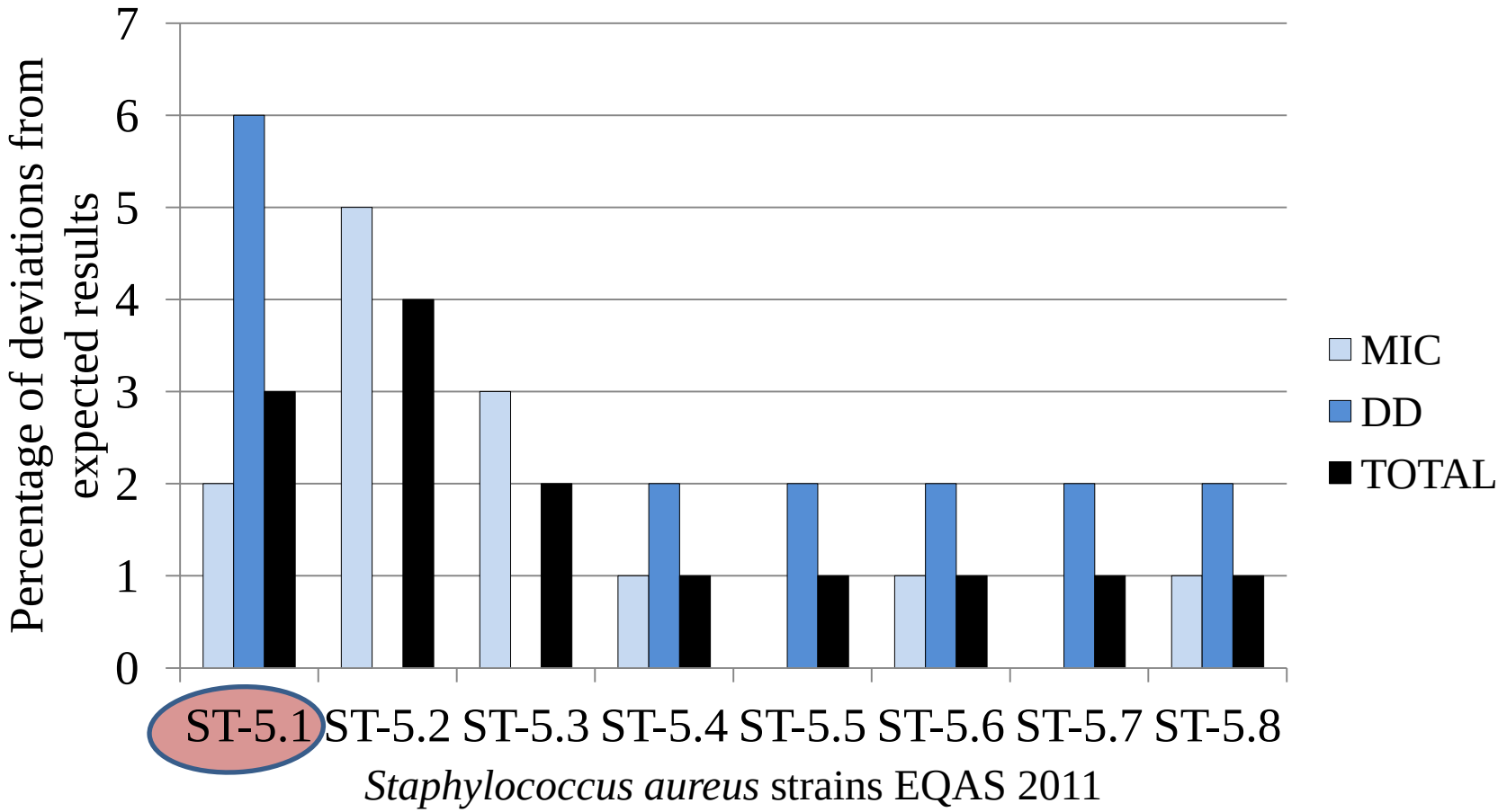
Staphylococci trial

Results omitted:

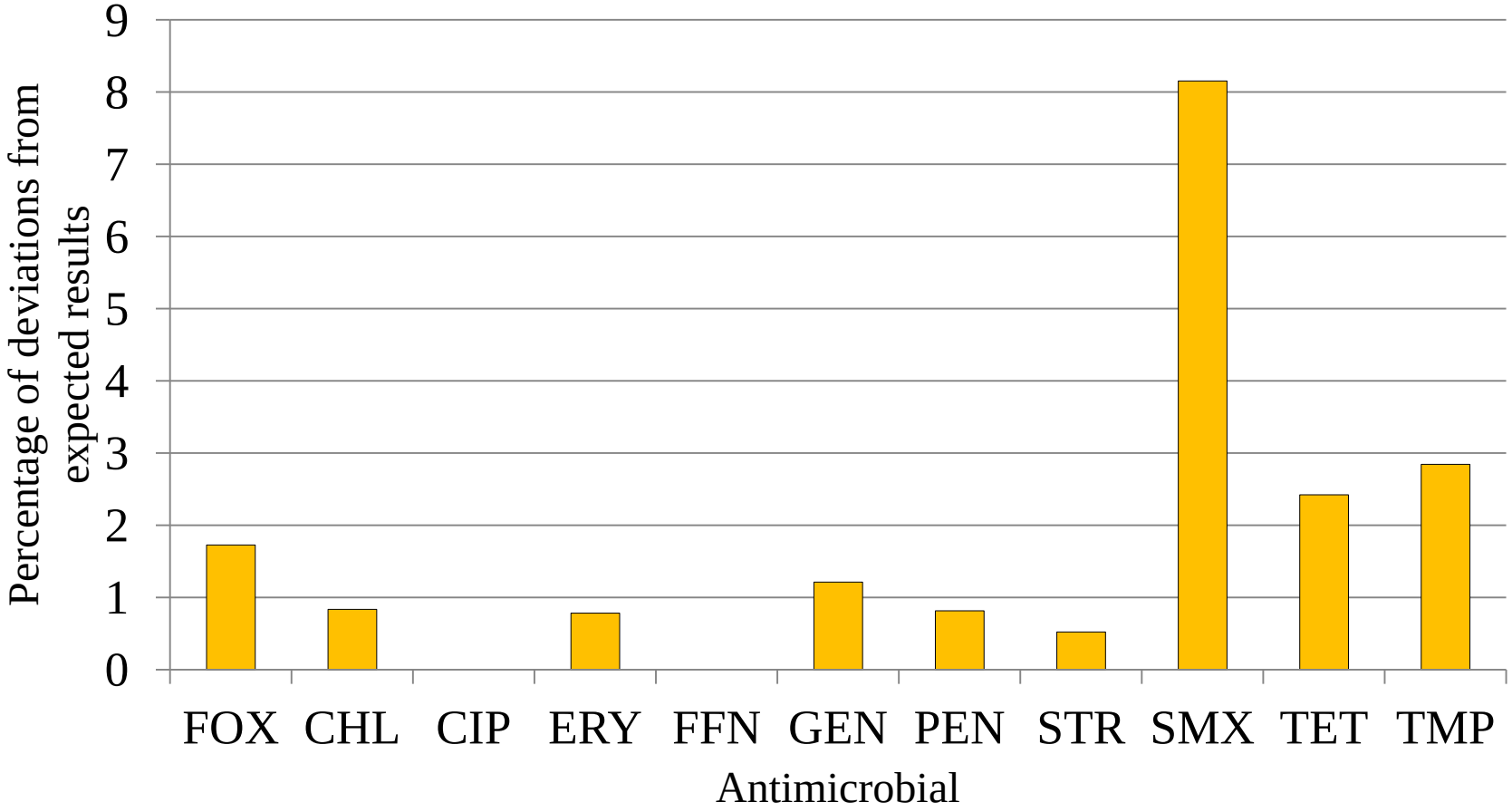
ST-5.1/CIP



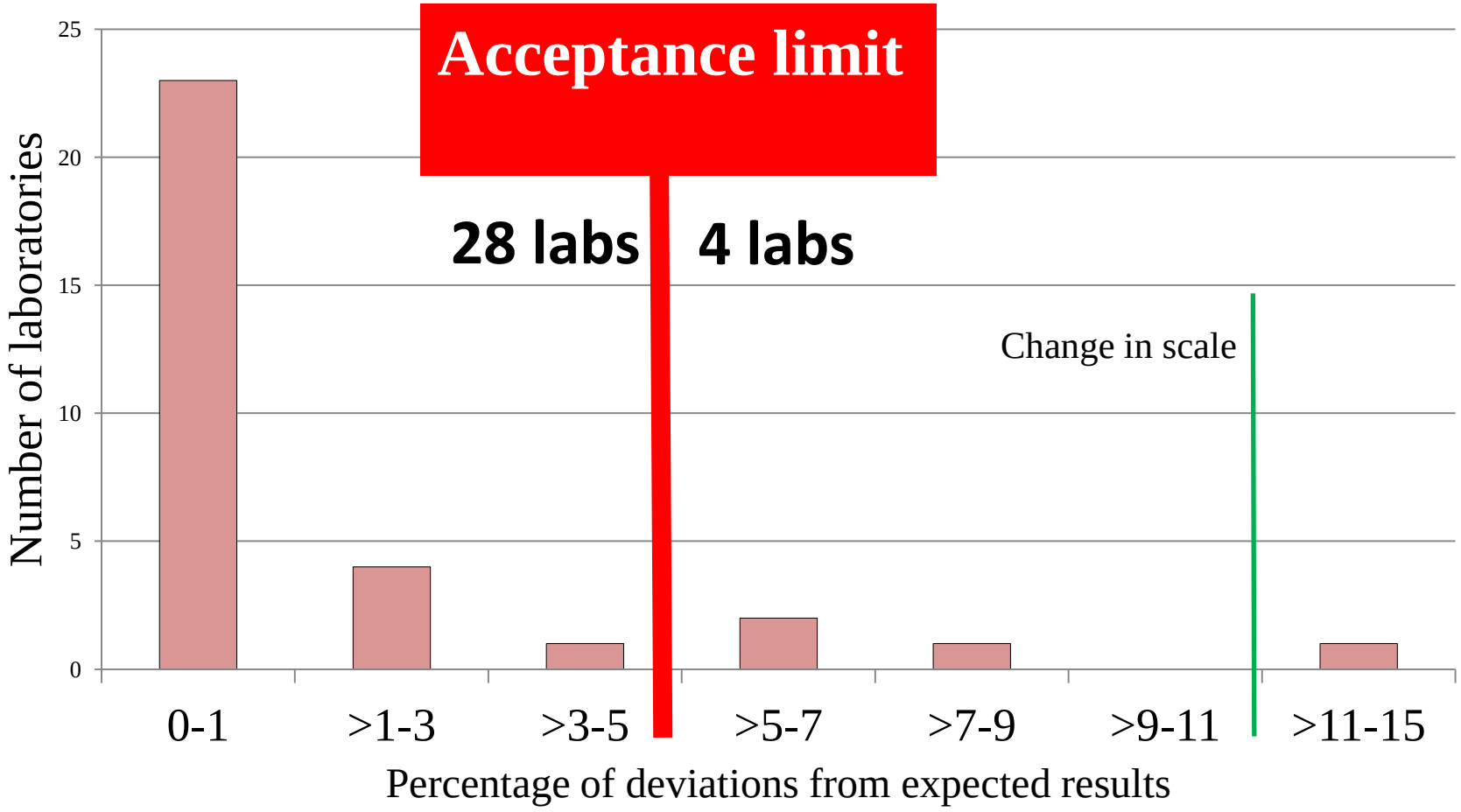
Deviations from expected results by strain tested and AST method used



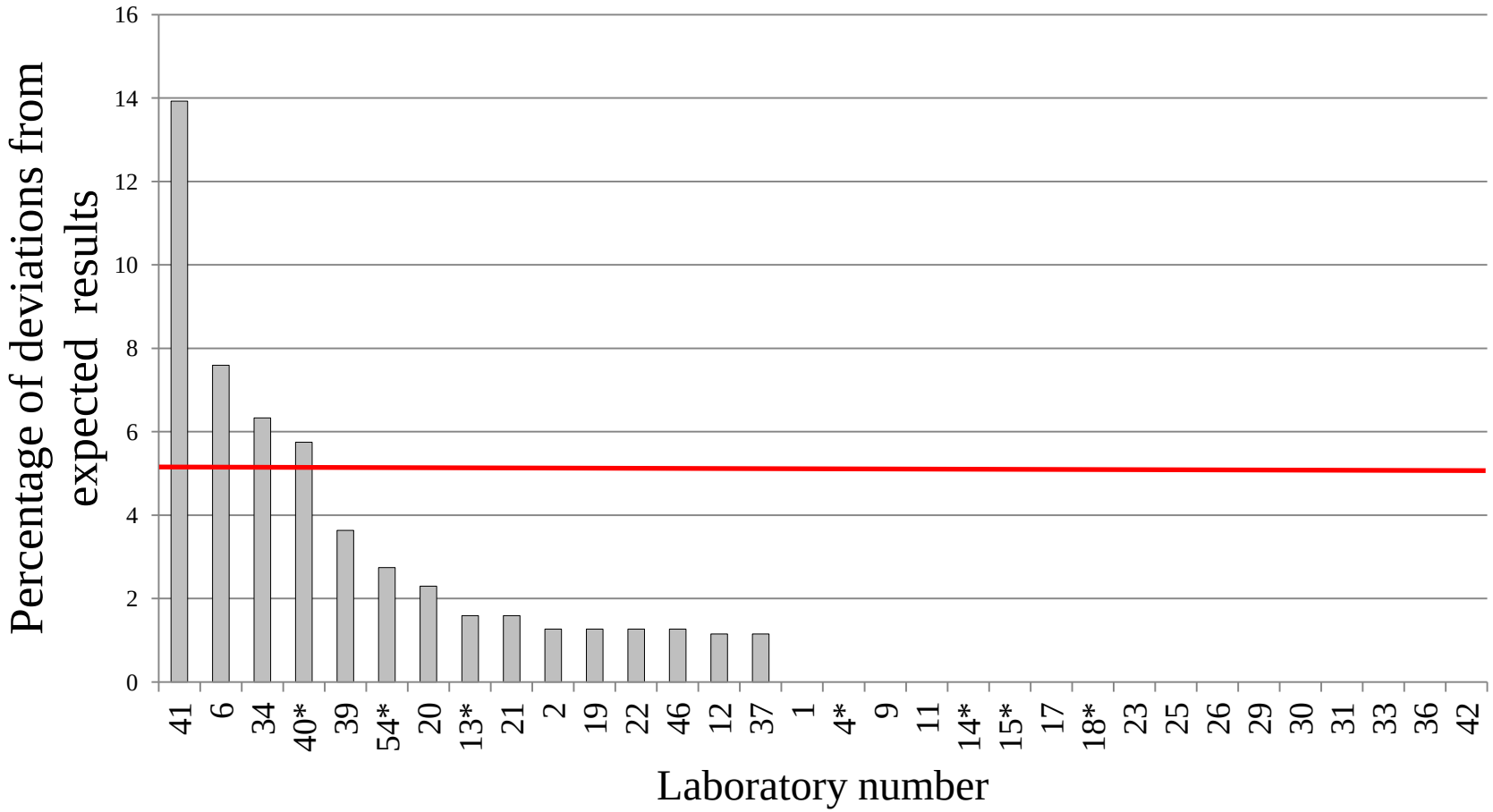
Deviations from expected results by antimicrobial tested



Overall lab. performance in staphylococci trial



Overall lab. performance in staphylococci trial



Methicillin-resistant *S. aureus* (MRSA)

- Strains ST-5.1, ST-5.5 and ST-5.8 were MRSA
- Of 32 participants testing staphylococci strains, one (# 41) did not report results concerning methicillin resistance
- Participant # 18 correctly reported ST-5.1 and ST-5.8 as methicillin-resistant but did not report any result for ST-5.5
- Participant # 4 reported strain ST-5.8 as methicillin-susceptible

Quality Control

Staphylococcus aureus ATCC 25923 (DD)

Antimicrobial	QC range (DD)	Min. value	Max. value	No. of deviations from expected result/Total no. of tests
Cefoxitin	23 - 29	26	**33**	**1/6**
Chloramphenicol	16 - 26	21	**27**	**1/4**
Ciprofloxacin	22 - 30	22	30	0/4
Erythromycin	22 - 30	23	30	0/5
Florfenicol	n. a.	22	27	n. a.
Gentamicin	19 - 27	19	**28**	**1/5**
Penicillin	26 - 37	32	**40**	**2/5**
Streptomycin	14 - 22	15	19	0/3
Sulfisoxazole	24 - 30	24	29	0/3
Tetracycline	24 - 34	28	32	0/4
Trimethoprim	19 - 26	20	24	0/2

Quality Control
Staphylococcus aureus ATCC 25913 (MIC)

Antimicrobial	QC range (MIC)	Min. value	Max. value	No. of deviations from expected result/Total no. of tests
Cefoxitin	1 - 4	1	4	0/19
Chloramphenicol	2 - 8	2	8	0/23
Ciprofloxacin	0.12 - 0.5	0.25	0.5	0/23
Erythromycin	0.25 - 1	0.25	1	0/24
Florfenicol	2 - 8	4	8	0/9
Gentamicin	0.125 - 1	0.25	≤ 2	0/23
Penicillin	0.25 - 2	**0.12**	2	**1/24**
Sulfisoxazole	32 - 128	64	128	0/12
Tetracycline	0.125 - 1	0.25	1	0/24
Trimethoprim	1 - 4	1	4	0/23

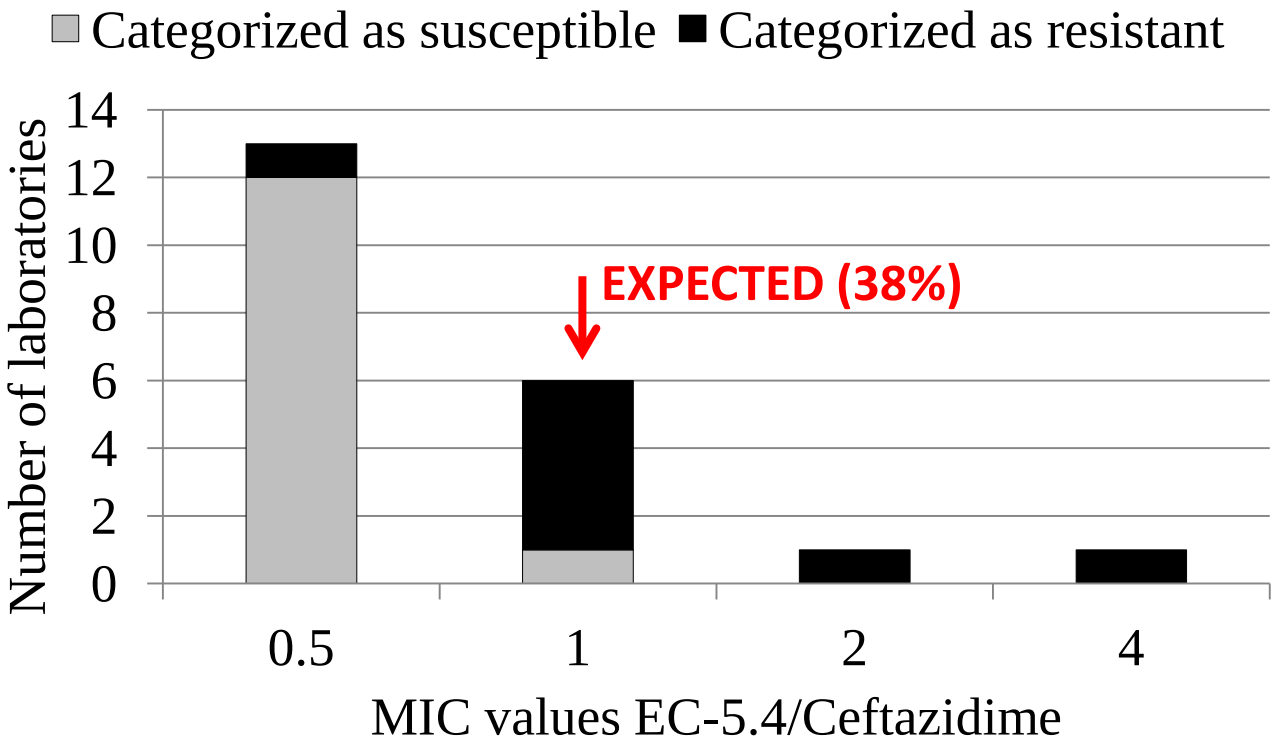
Conclusions (staphylococci)

- 32 participants (25: MIC; 7: DD)
- 4 laboratories outside the acceptance limit
- Good performance in MRSA detection
- Test of QC strains quite satisfactory

Escherichia coli trial

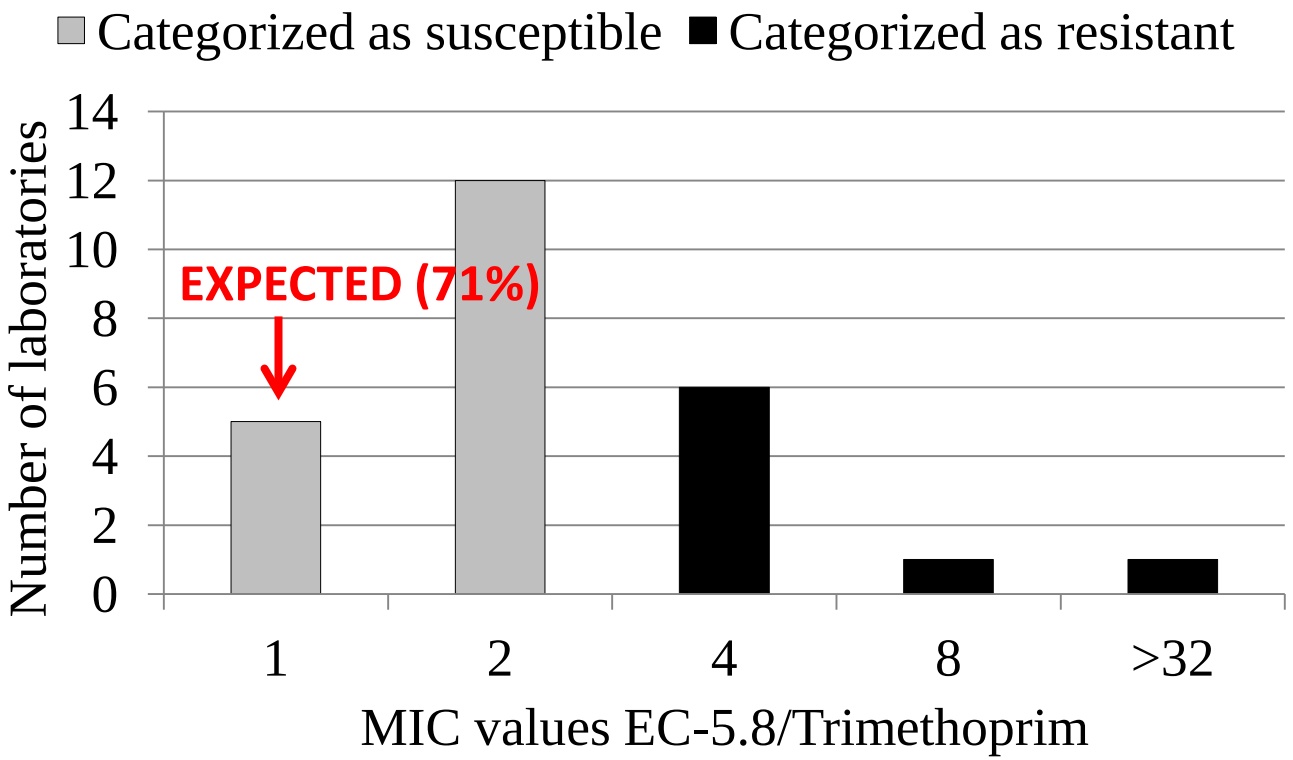
Results omitted:

EC-5.4/CAZ

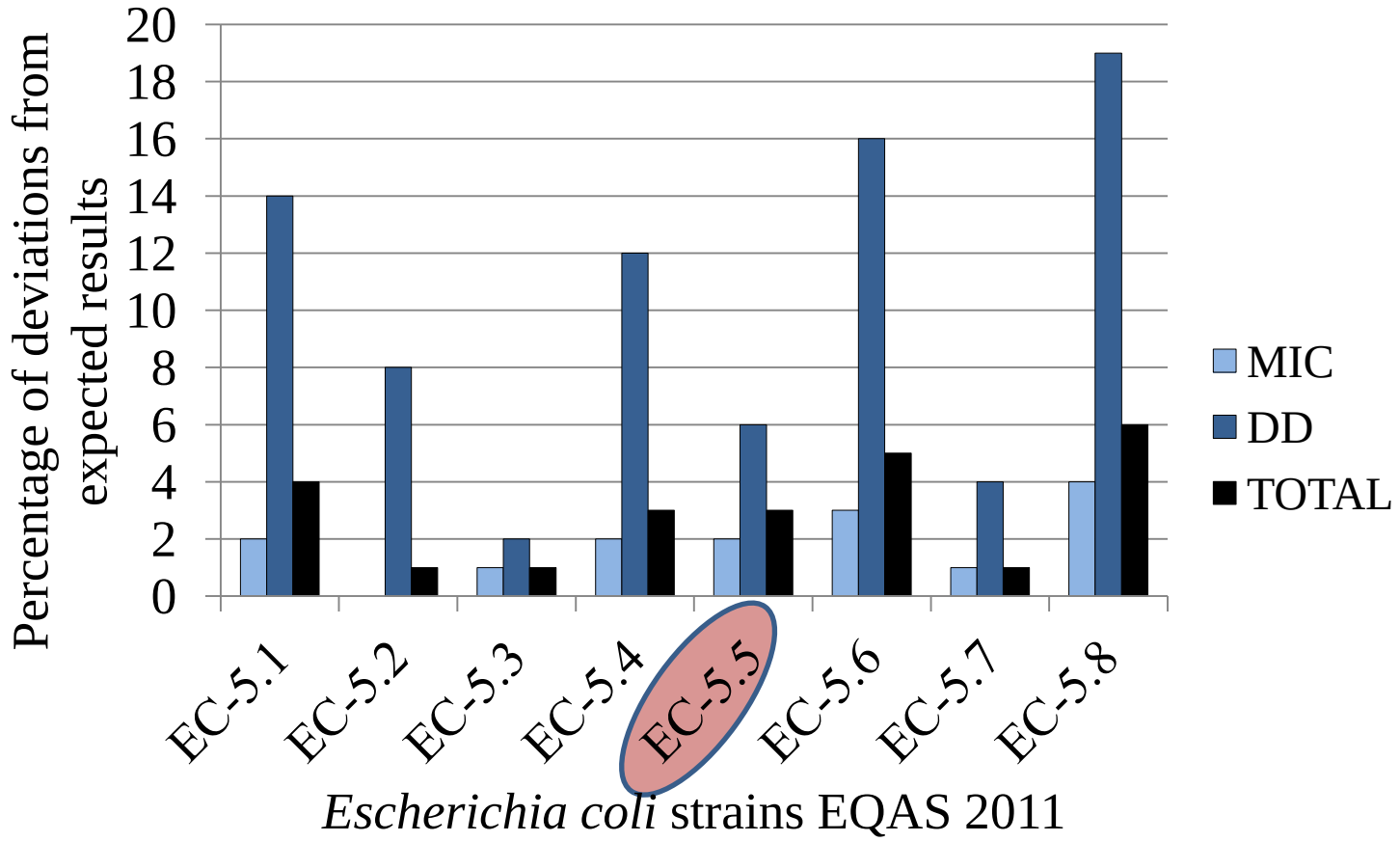


Results not omitted:

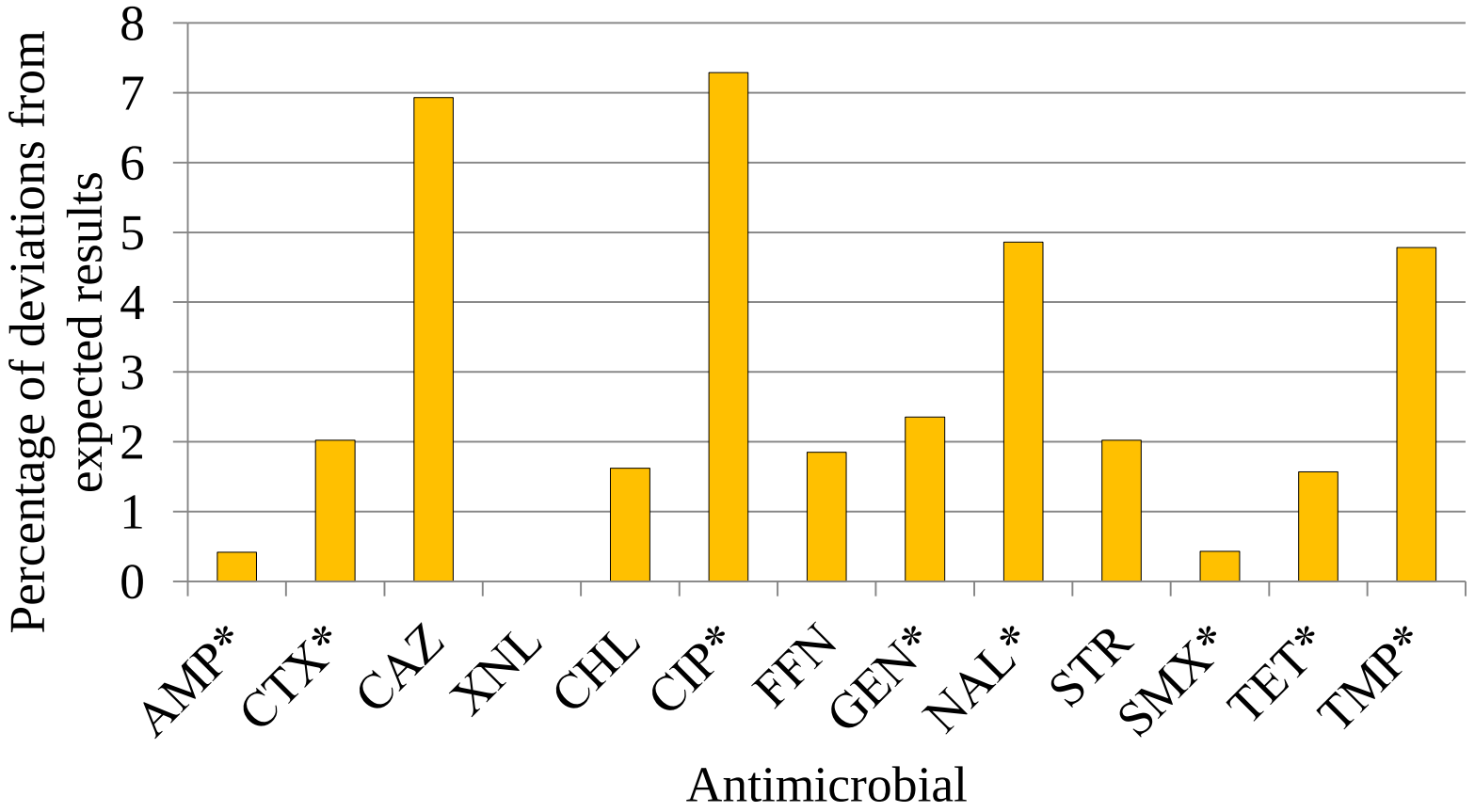
EC-5.8/TMP



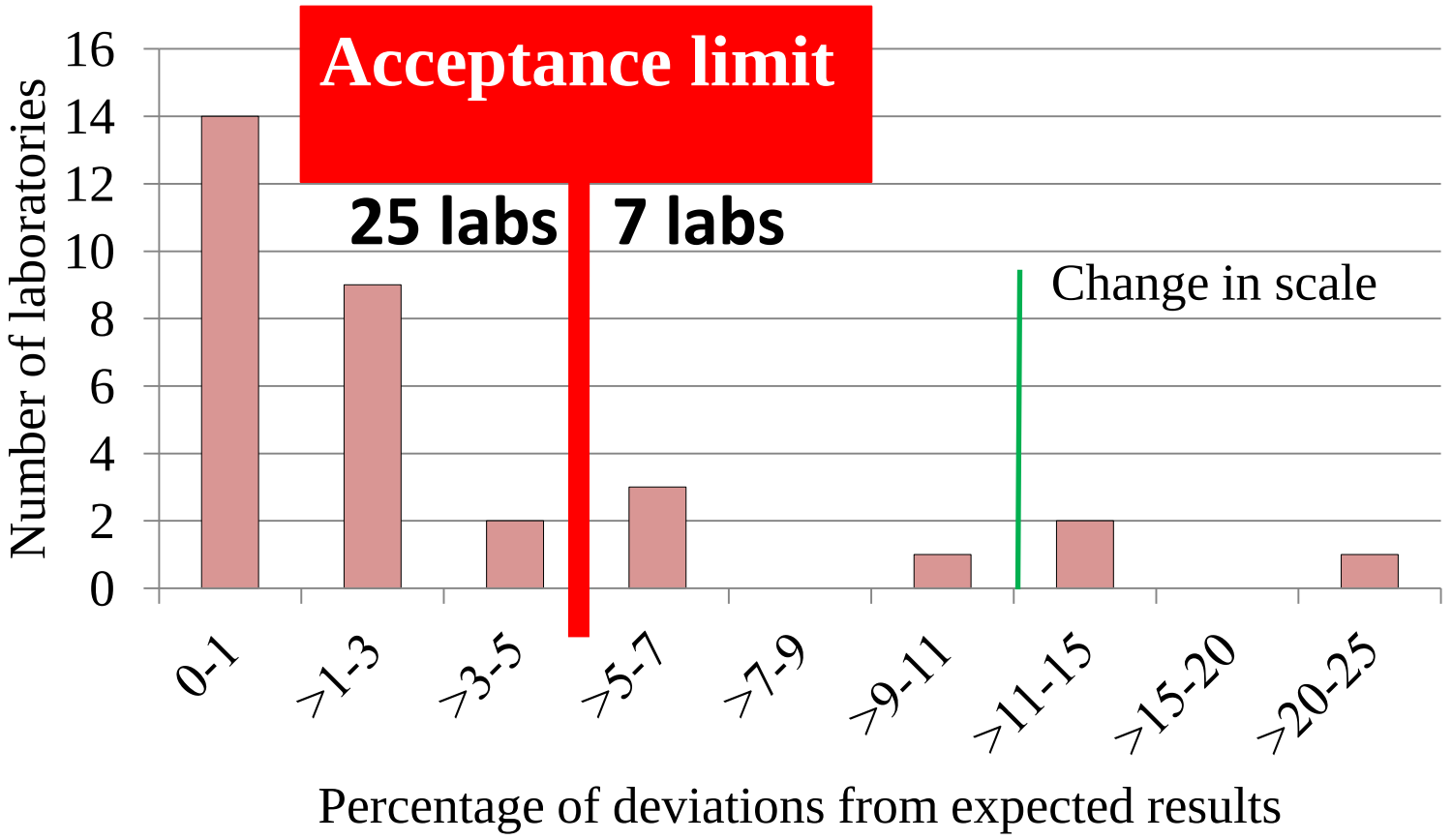
Deviations from expected results by strain tested and AST method used



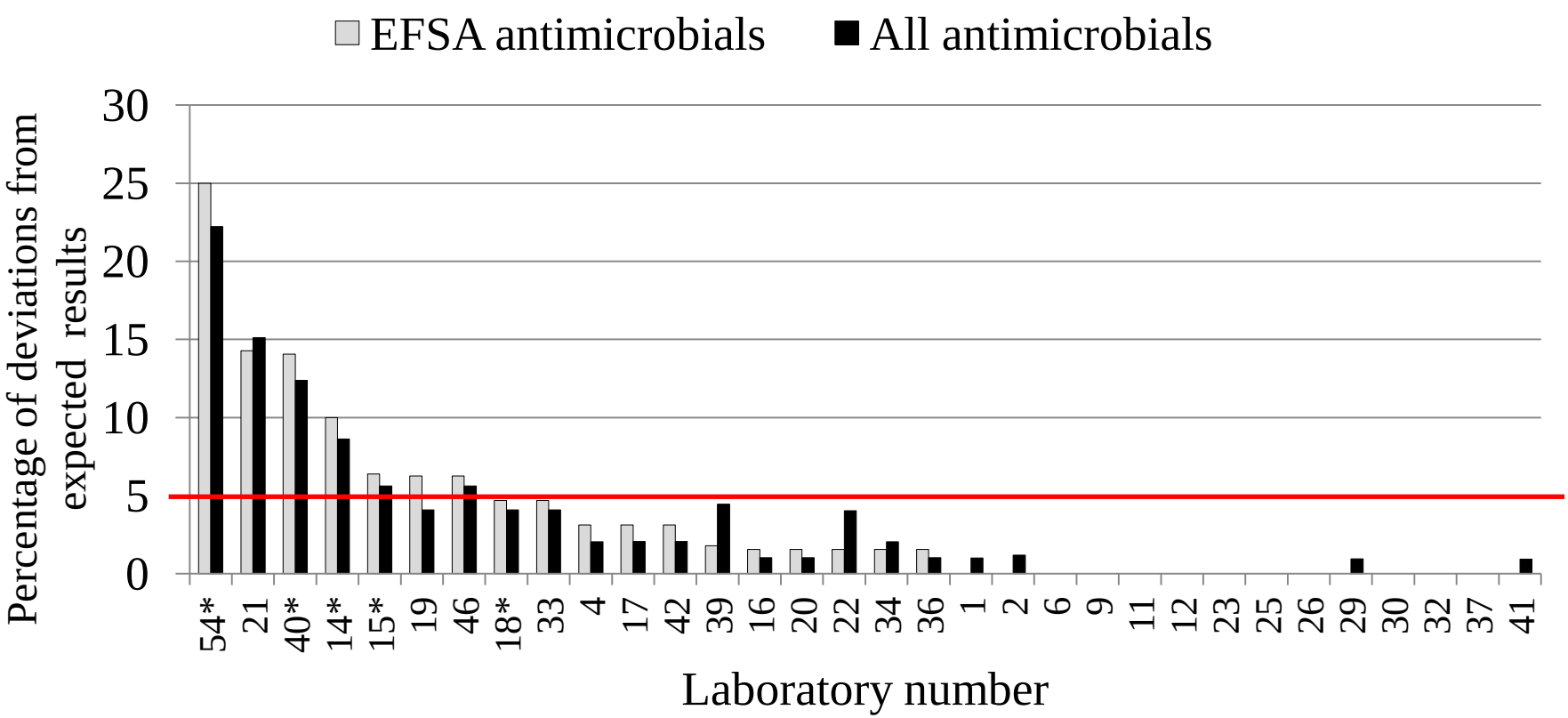
Deviations from expected results by antimicrobial tested



Overall lab. performance in *E. coli* trial



Overall lab. performance in *E. coli* trial



Detection of beta-lactamases (ESBL and AmpC-type)

EC-5.4 and EC-5.5 were extended-spectrum beta-lactamase (ESBL) producers and EC-5.8 was an AmpC-producer

Detection of beta-lactamases (ESBL and AmpC-type): how to…

- Initial test with ≥ 2 third-generation cephalosporins
  (e.g. cefotaxime, ceftazidime, ceftiofur, cefpodoxime,…)

  The use of more the one compound increase the sensitivity (genetic basis: ≠ genes -> ≠ enzymes -> ≠ affinity for relatively similar antimicrobial compounds)

- **IF RESISTANT** to the above, then **confirmatory test**
  (cephalosporin & combination cephalosporin+inhibitor, e.g.: CTX & CTX+CLA and CAZ & CAZ+CLA)

  The confirmatory test is used to detect possible synergy:

  a ≥ 3 two-fold reduction in MIC (e.g. from 8 to 1 µg/ml) or a ≥ 5mm increase in a zone diameter between CAZ and CAZ/CLA or CTX and CTX/CLA

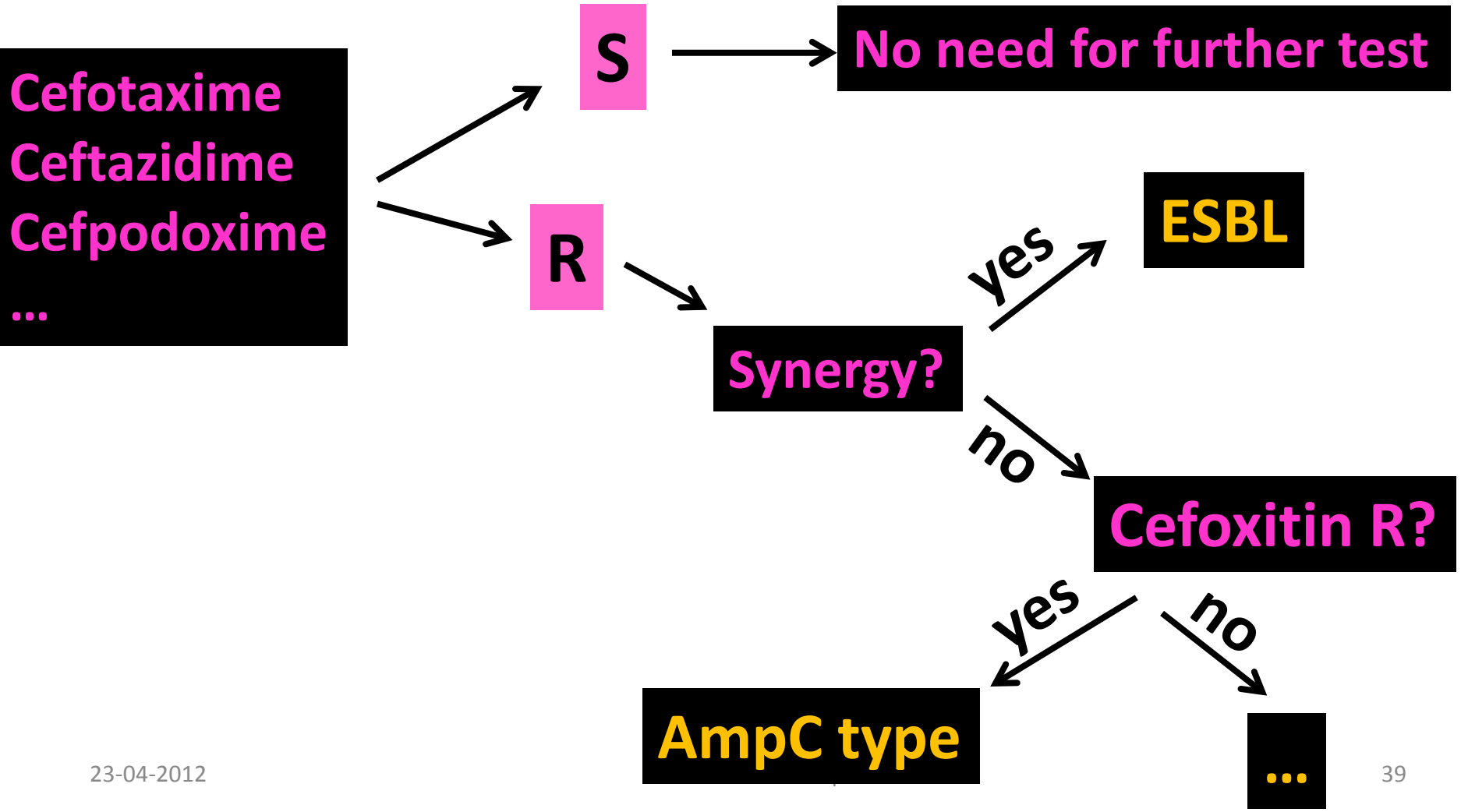
Detection of beta-lactamases (ESBL and AmpC-type): how to…

- IF SYNERGY: **ESBL**

- IF NO SYNERGY: **AmpC-type?**

  Test for cefoxitin resistance (FOX resistant)

Detection of beta-lactamases (ESBL and AmpC-type): **FLOW DIAGRAM**



Detection of beta-lactamases (ESBL and AmpC-type)

- Participant #54 did not report results for this component
- # 22, # 46 and did not identify EC-5.4 as an ESBL producer as they did not obtained signs of synergy by testing cefotaxime and cefotaxime+clavulanic acid

Detection of beta-lactamases (ESBL and AmpC-type)

- # 21 and # 22 erroneously classified strain EC-5.6 as ESBL producer: # 21 erroneously categorized the strain as cefotaxime and ceftazidime resistant, performed confirmatory test and found synergy by testing cefotaxime and cefotaxime+clavulanic acid; # 22 performed confirmatory test despite having correctly categorized EC-5.6 as cefotaxime and ceftazidime susceptible and classified the strain as ESBL producer due to “fake synergy”

Detection of beta-lactamases (ESBL and AmpC-type)

- Ten participants did not identify strain EC-5.8 as AmpC producer
- # 41 did not identify EC-5.8 as AmpC producer and classified it instead as ESBL producer (phantom area)
- # 22 classified EC-5.8 as ESBL and AmpC-producer

Quality Control
Escherichia coli ATCC 25922 (DD)

Antimicrobial	QC range (DD)	Min. value	Max. value	No. of deviations from expected result/Total no. of tests
Ampicillin	16 - 22	19	20	0/2
Cefotaxime	29 - 35	30	35	0/4
Cefoxitin	23 - 29	26	28	0/4
Ceftazidime	25 - 32	26	32	0/3
Ceftiofur	26 - 31	26	29	0/3
Chloramphenicol	21 - 27	22	26	0/3
Ciprofloxacin	30 - 40	35	38	0/3
Gentamicin	19 - 26	20	26	0/4
Imipenem	26 - 32	30	30	0/1
Nalidixic acid	22 - 28	25	26	0/4
Streptomycin	12 - 20	16	18	0/2
Sulfisoxazole	15 - 23	21	23	0/2
Tetracycline	18 - 25	24	**27**	**1/4**
Trimethoprim	21 - 28	26	28	0/3

Quality Control
Escherichia coli ATCC 25922 (MIC)

Antimicrobial	QC range (MIC)	Min. value	Max. value	No. of deviations from expected result/Total no. of tests
Ampicillin	2 - 8	2	8	0/26
Cefotaxime	0.03 - 0.125	0.06	0.125	0/26
Cefoxitin	2 - 8	2	8	0/8
Ceftazidime	0.06 - 0.5	0.25	**1**	**1/23**
Ceftiofur	0.25 - 1	0.25	0.5	0/4
Chloramphenicol	2 - 8	2	8	0/26
Ciprofloxacin	0.004 - 0.016	0.008	**0.06**	**3/26**
Gentamicin	0.25 - 1	0.25	**2**	**2/26**
Imipenem	0.06 - 0.25	0.12	0.5	0/4
Nalidixic acid	1 - 4	2	4	0/26
Streptomycin	4 - 16	4	8	0/25
Sulfisoxazole	8 - 32	8	**128**	**2/17**
Tetracycline	0.5 - 2	1	2	0/25
Trimethoprim	0.5 - 2	0.5	2	0/25

Conclusions (*E. coli*)

- 32 participants (27: MIC; 5: DD)
- 7 laboratories outside the acceptance limit
- Good performance in ESBL detection
- Improvement is needed in AmpC detection
- Test of QC strains is satisfactory

Some general considerations…

- QC strains should be tested by all participants
- Few interpretation errors… this can easily be corrected
- Systematic errors? If identified, corrective actions can be successfully implemented
- You are welcome to contact me during the workshop for individual discussion

Thank you for your attention