



EQAS 2009

Enterococci, Staphylococci *and E. coli*

EURL workshop, April 8, 2010

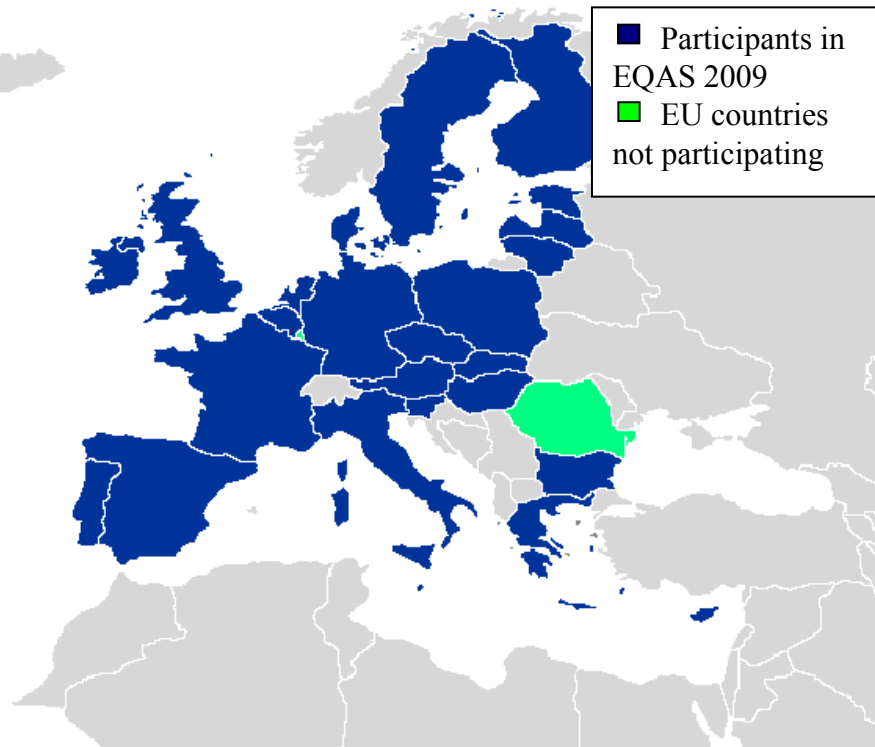
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Main objectives of the EURL EQAS's

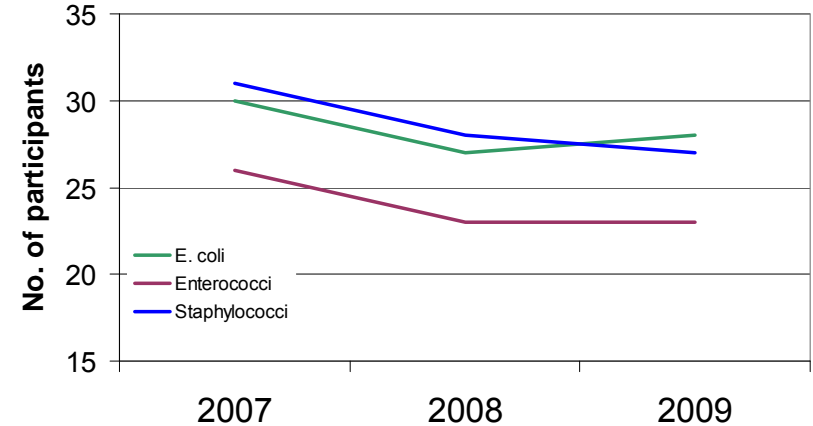
- To improve the comparability of antimicrobial susceptibility testing (AST) data
- To harmonise the breakpoints/epidemiological cut off values
- To assess the quality of AST in European laboratories and identify possible barriers
- To support laboratories in performing, evaluating and if necessary improving the quality of AST



Participants in the enterococci, staphylococci and *E. coli* EQAS, 2009



Number of participating labs



Methods for EQAS 2009

- Eight strains of enterococci, staphylococci and *E. coli*, respectively were selected
- New participants were provided with the reference strains, *E. faecalis* ATCC 29212, *S. aureus* ATCC 25923, *S. aureus* ATCC 29213 and *E. coli* ATCC 25922 for QC testing
- AST guidelines were set according to the CLSI. MIC results were interpreted using the epidemiological cut off values set by EUCAST (www.eucast.org), recommended by EFSA and described in the protocol
- Participants using disk diffusion were advised to interpret the results according to their individual breakpoints
- Results were categorized as resistant or susceptible

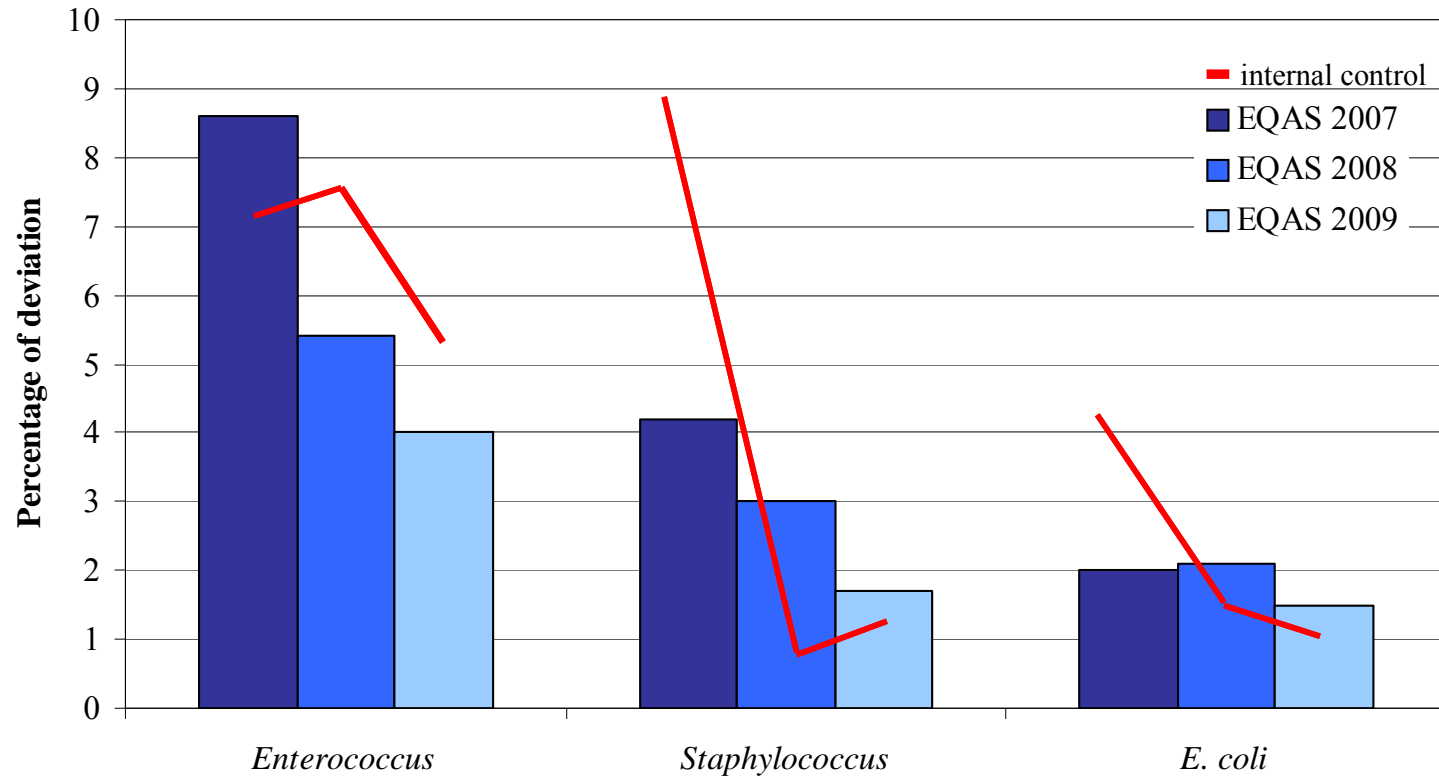


Analysis of data based on these agreements

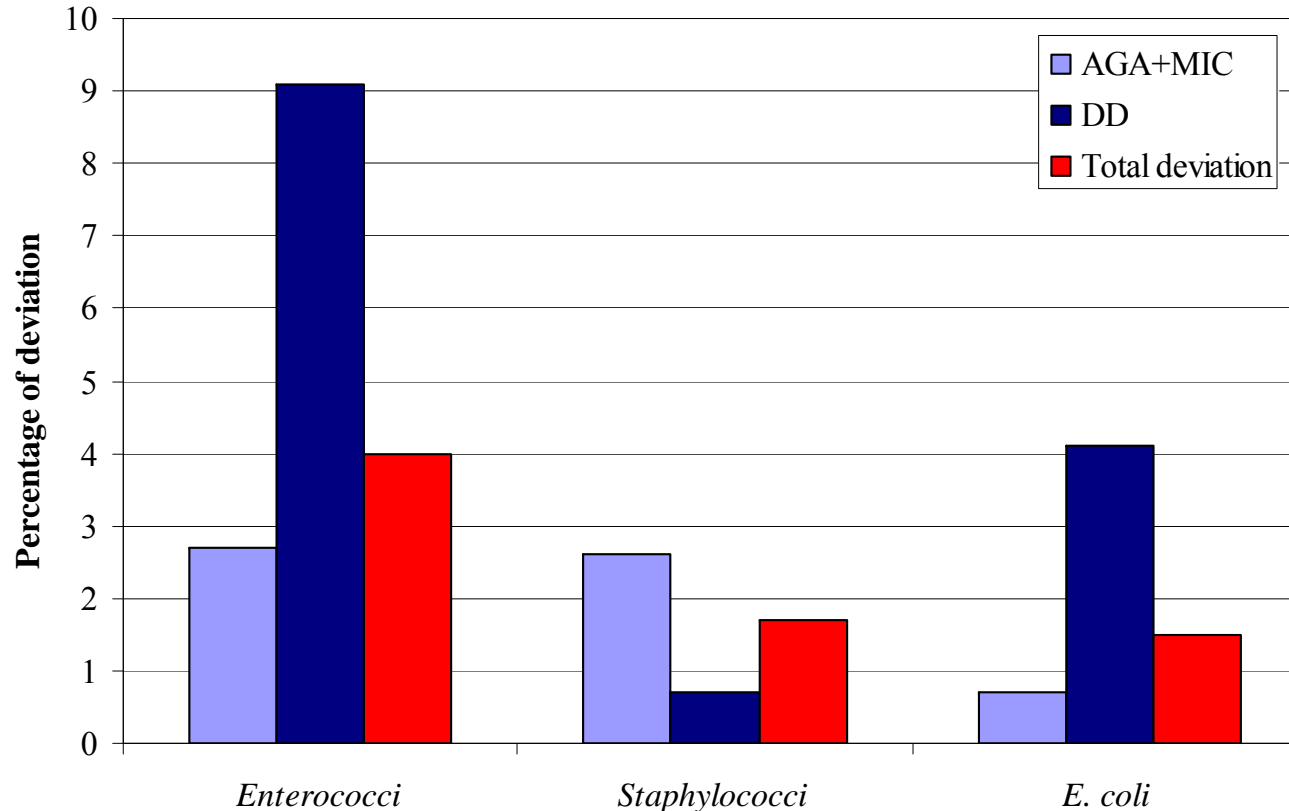
- During the passed EURL-AR Workshop (2008) the network agreed upon the following decisions for EQAS 2009:
 - The accepted deviation for each laboratory was set up at 5% instead of 7%
 - Results should be further analysed (possibly ignored) if only 75% are correct (test strain/antimicrobial combination)



EQAS 2009 versus previous EQAS



Deviation by species comparing the AST methods



- Significant differences were observed in the results obtained depending of the AST method used ($p < 0.01$)

Review of antimicrobials tested

Enterococci trial	Staphylococci trial*	<i>E. coli</i> trial
Ampicillin [†]	Cefoxitin	Ampicillin [†]
Avilamycin	Chloramphenicol	Cefotaxime [†]
Chloramphenicol [†]	Ciprofloxacin	Ceftazidime
Ciprofloxacin	Erythromycin	Ceftiofur
Daptomycin	Florfenicol	Chloramphenicol [†]
Erythromycin [†]	Gentamicin	Ciprofloxacin [†]
Gentamicin [†]	Penicillin	Florfenicol
Linezolid [†]	Streptomycin	Gentamicin [†]
Streptomycin [†]	Sulfonamides	Nalidixic acid [†]
Quinupristin-dalfopristin [†]	Tetracycline	Streptomycin [†]
Tetracycline [†]	Trimethoprim	Sulphonamides [†]
Tigecycline		Tetracycline [†]
Vancomycin [†]		Trimethoprim [†]

Enterococci trial - results

- Results that have been omitted from the evaluation

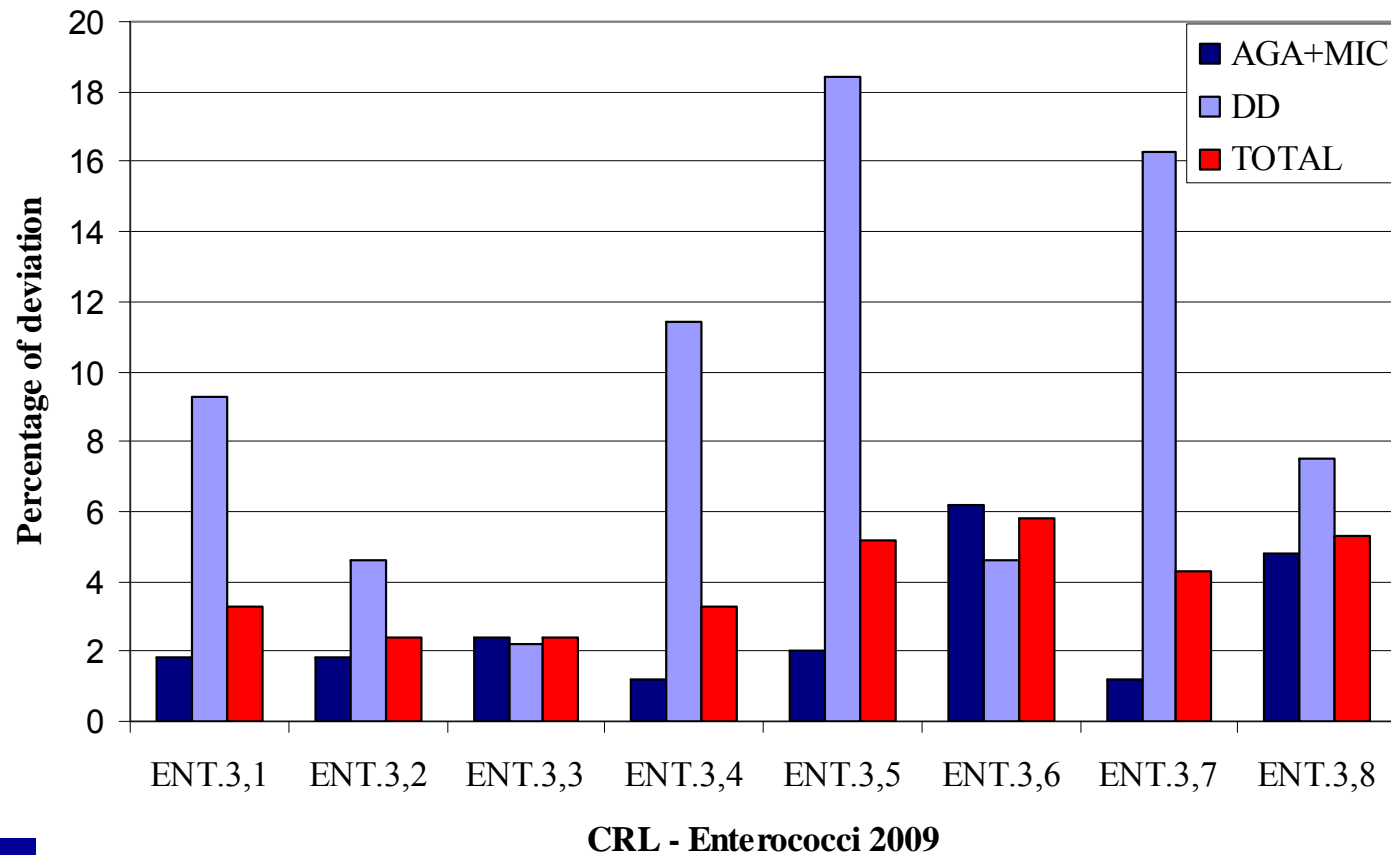
Strain	Antimicrobial	Correct R/S	Percentage correct results	Expected MIC	Cut off Value (R >)	Deviations MIC/n ¹	Deviations DD/n ²
ENT.3,5	Ciprofloxacin	R	41%	>4	4	10/12	0/5
ENT.3,5	Daptomycin	R	33%	8	4	2/3	0/0
ENT.3,6	Daptomycin	S	33%	4	4	2/3	0/0

Lab no	strain	antimicrobial	Results	MIC	Expected	Expected
19	CRL ENT.3,5	Ciprofloxacin	R	4	R	>4
24			R	8		
2			S	4		
9			S	4		
12			S	4		
17			S	4		
20			S	4		
21			S	4		
25			S	4		
34			S	4		
37			S	4		



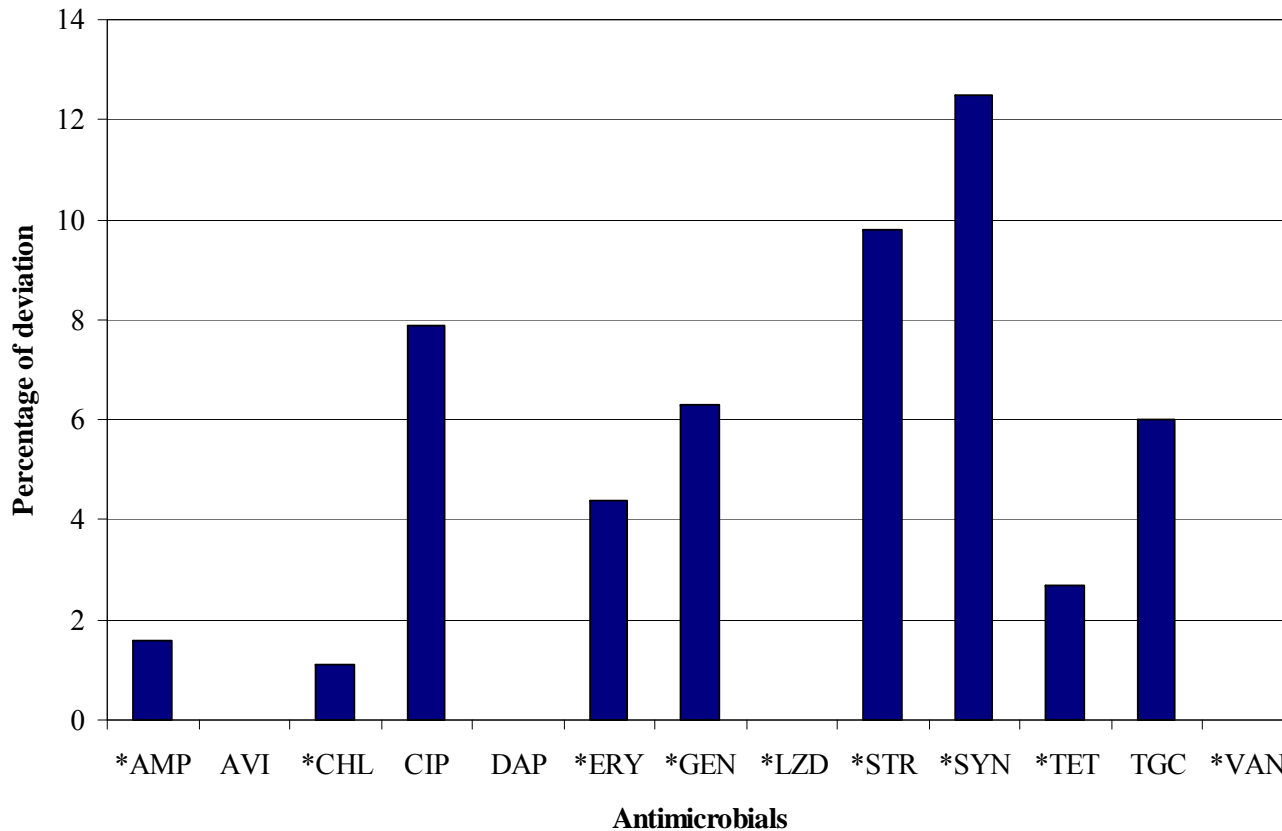
Enterococci trial - results

- Deviation by strain and AST method



Enterococci trial - results

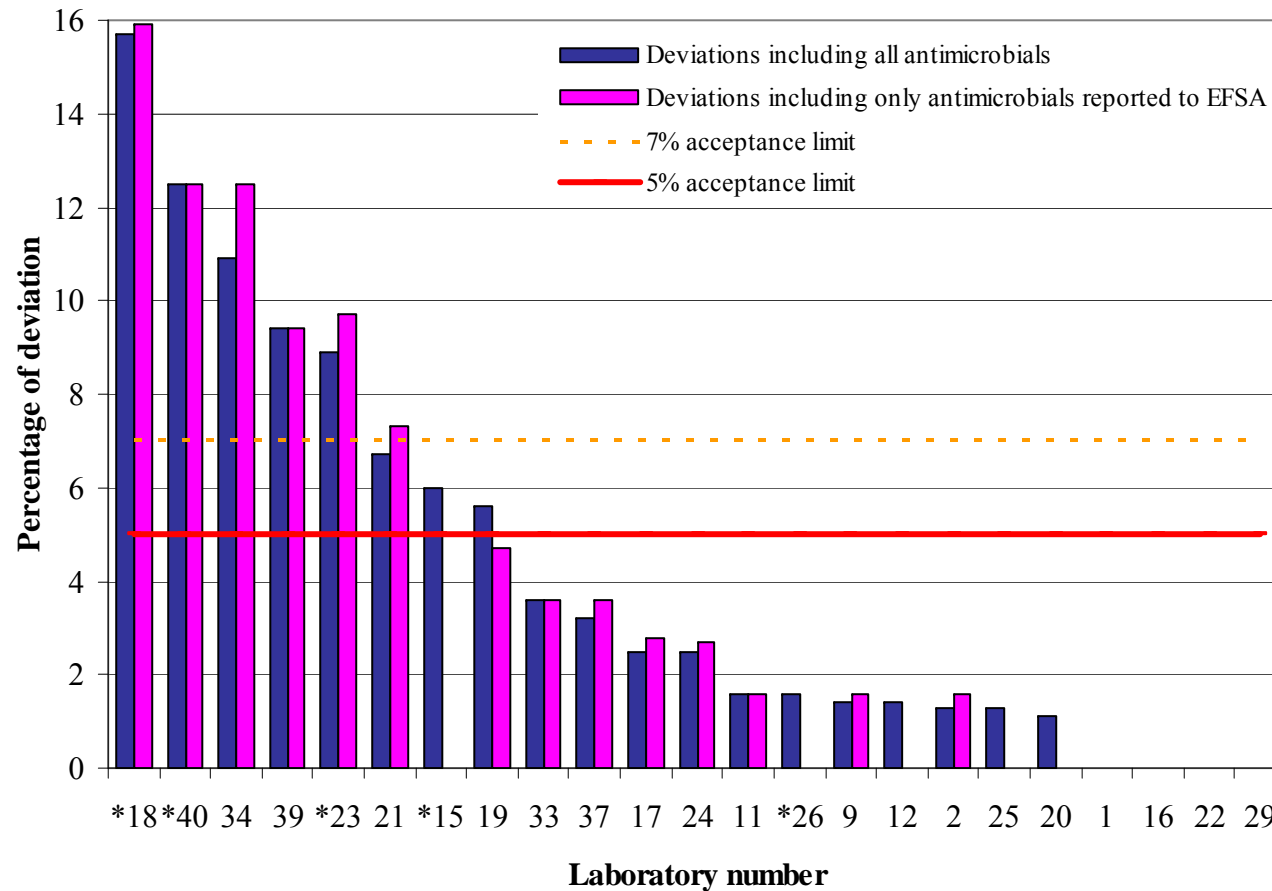
- Deviation by antimicrobial tested



*Antimicrobials recommended by EFSA for monitoring antimicrobial resistance across the EU

Enterococci trial - results

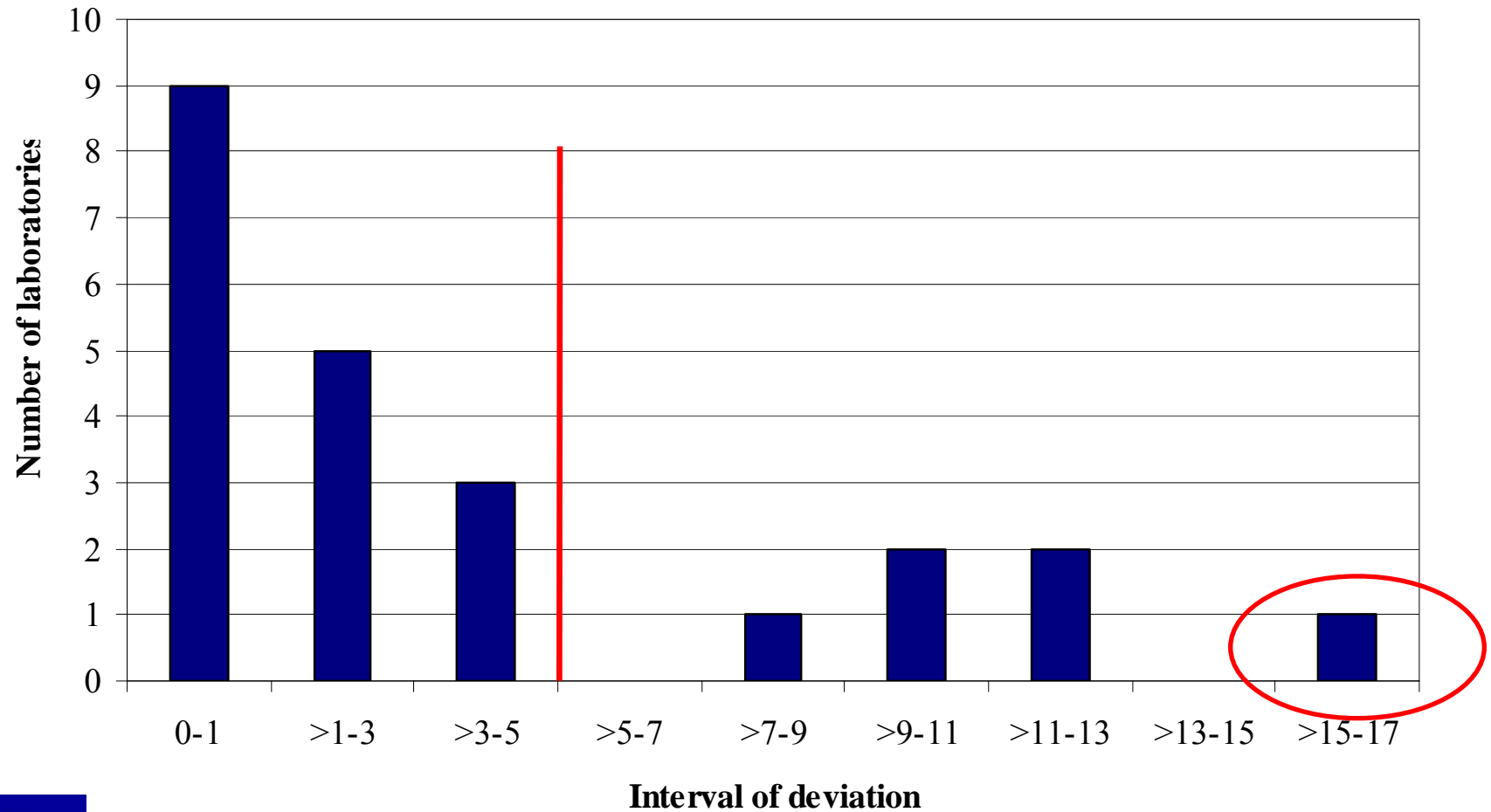
- Deviation by laboratory



*Laboratories performing DD for AST



Enterococci trial - results



17 labs

6 labs



QC- STRAIN MIC

- 17 participants
- **138/139** correct tests performed

<i>E. faecalis</i> ATCC 29212				
Antimicrobial	MIC deviations /Total no. of test	QC range MIC	Min value	Max value
Ampicillin	0/17	0.5 - 2	0.5	2
Avilamycin,	0/3	0.5 - 4	1	4
Chloramphenicol	0/17	4 - 16	4	8
Ciprofloxacin	0/10	0.25 - 2	0.5	2
Daptomycin	0/3	1 - 8	1	2
Erythromycin	0/17	1 - 4	1	4
Gentamicin	0/17	4 - 16	4	≤128
Linezolid	1/11	1 - 4	0.5	2
Synacid	0/8	2 - 8	4	8
Tetracycline	0/17	8 - 32	8	32
Tigecycline	0/3	0.03 - 0.12	0.12	0.1
Vancomycin	0/16	1 - 4	2	4

Summarizing enterococci trial

- Deviations in the eight enterococci strains fall below 6%
 - decreased in deviation caused by labs performing DD from 16% to 9.1% when compared to 2008
- 7/9 antimicrobials recommended by EFSA failed to produce 100% of correct results
 - High level resistance to aminoglycosides deviations caused by participants using DD
 - Synacid
- The number of laboratories deviating more than the 5% is still high (6/23)
- Deviations were mainly caused by laboratories performing DD for AST



Staphylococci trial - results

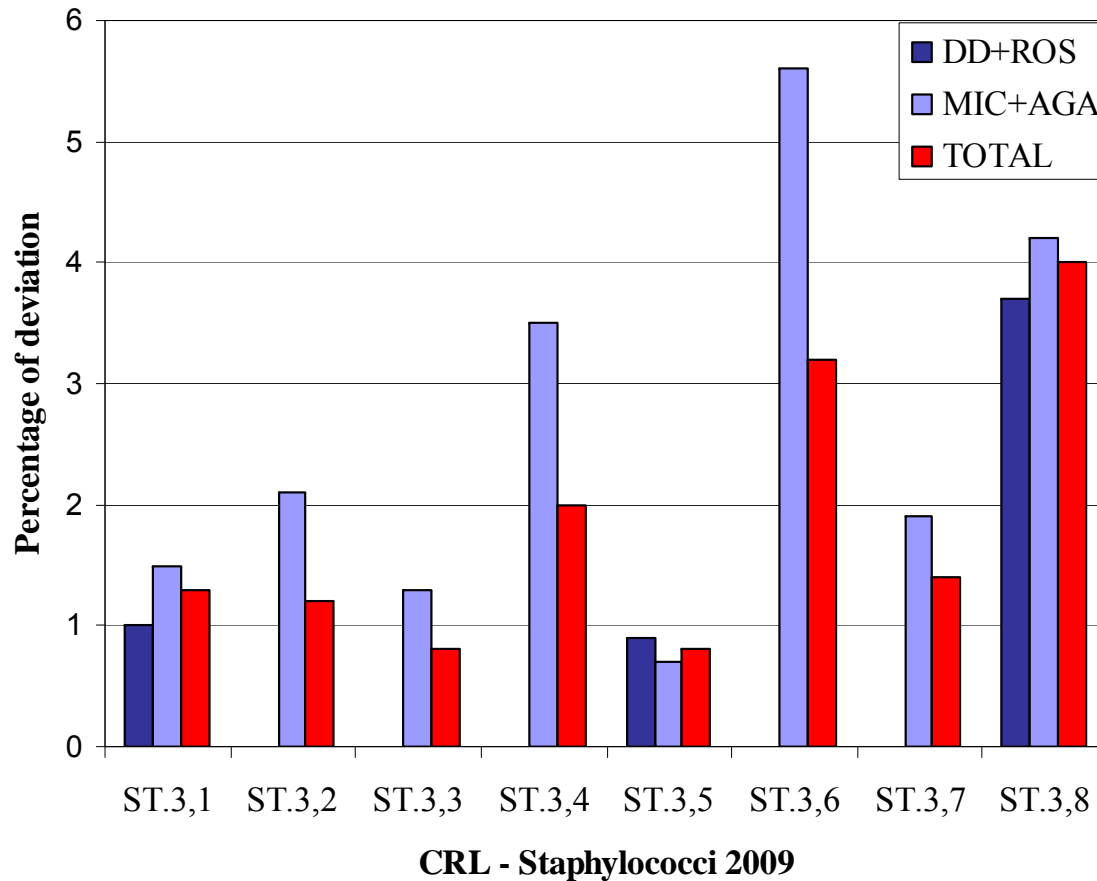
- Results that have been omitted from the evaluation

Strain	Antimicrobial	Correct R/S	Percentage correct results	Expected MIC	Cut off value (R >)	Deviations MIC/n ¹	Deviations DD/n ²
ST.3,1	Ciprofloxacin	R	33%	2	1	8/13	8/11

Lab no	strain	antimicrobial	Result	MIC	Expected	Expected
1	CRL ST.3,1	Ciprofloxacin	R	2	R	2
11			S	1		
12			S	1		
17			R	2		
19			S	1		
20			R	2		
22			S	1		
24			S	1		
26			R	2		
29			S	1		
31			R	>1		
34			S	1		
37			S	1		
39			S	<=0.25		

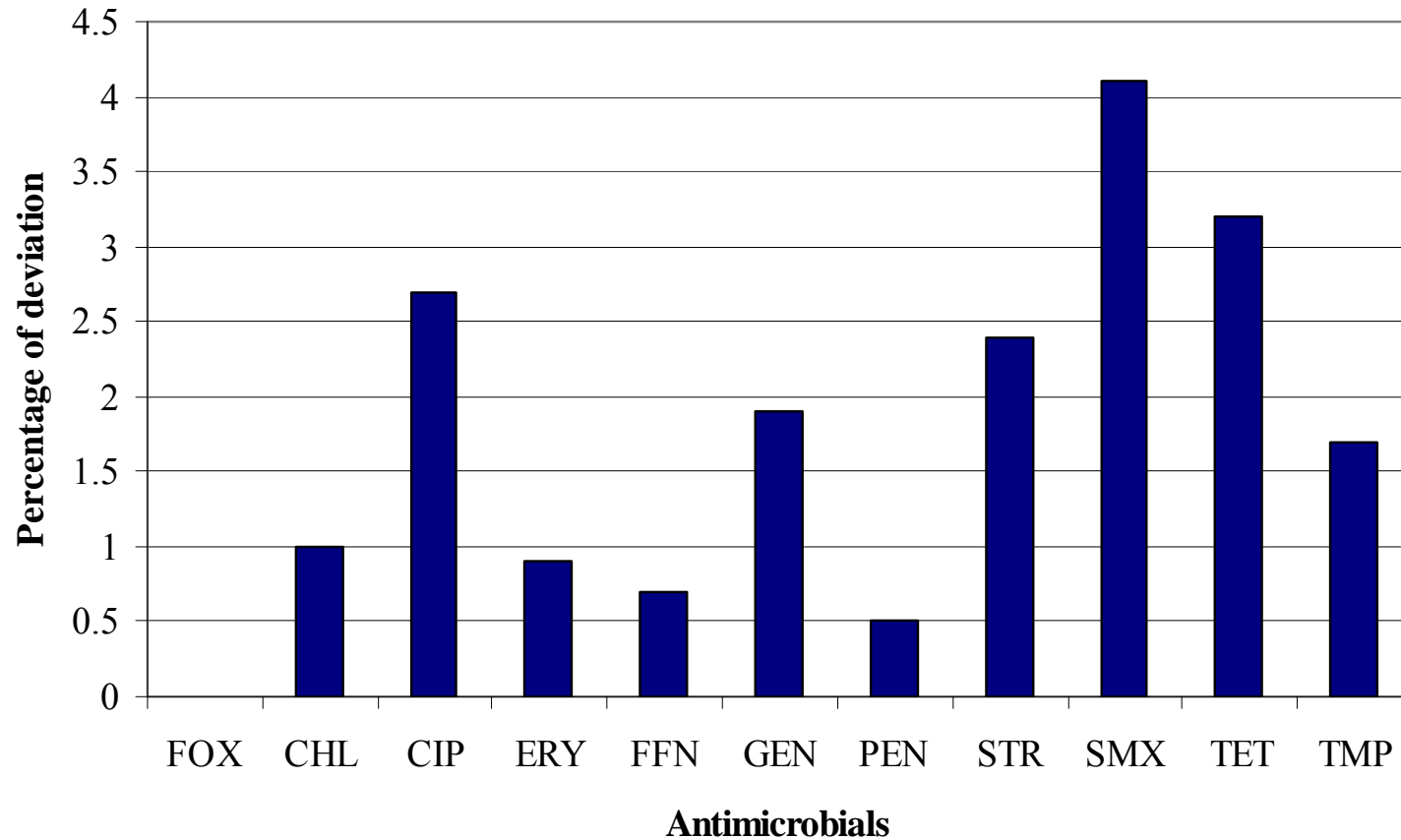
Staphylococci trial - results

- Deviation by strain



Significantly higher deviations were observed by participants performing MIC compared to disk diffusion methods ($p = 0.003$).

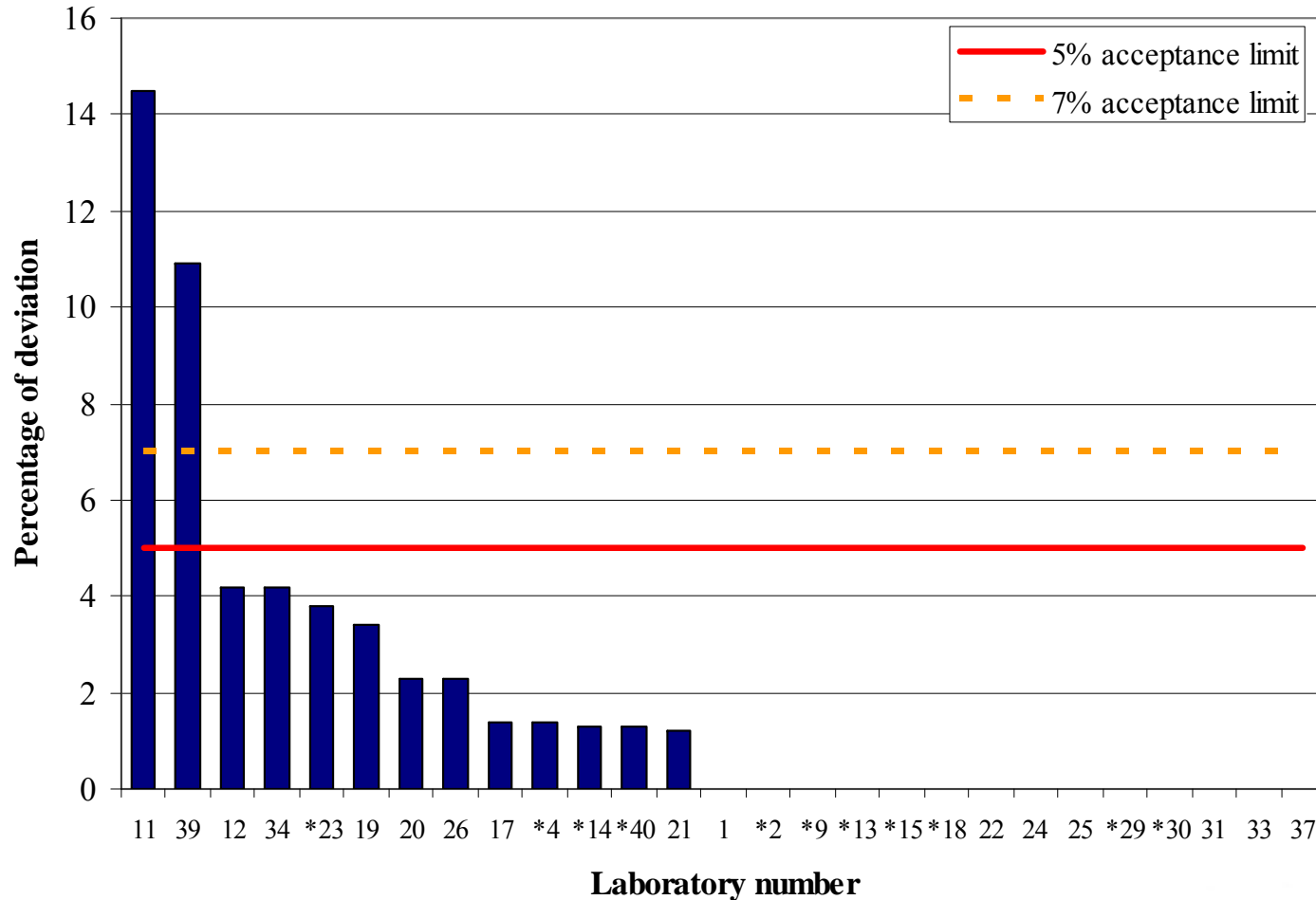
Staphylococci trial - results



Sulfamethoxazole has a bacteriostatic effect interpretation of results can be uncertain for both MIC and disk diffusion

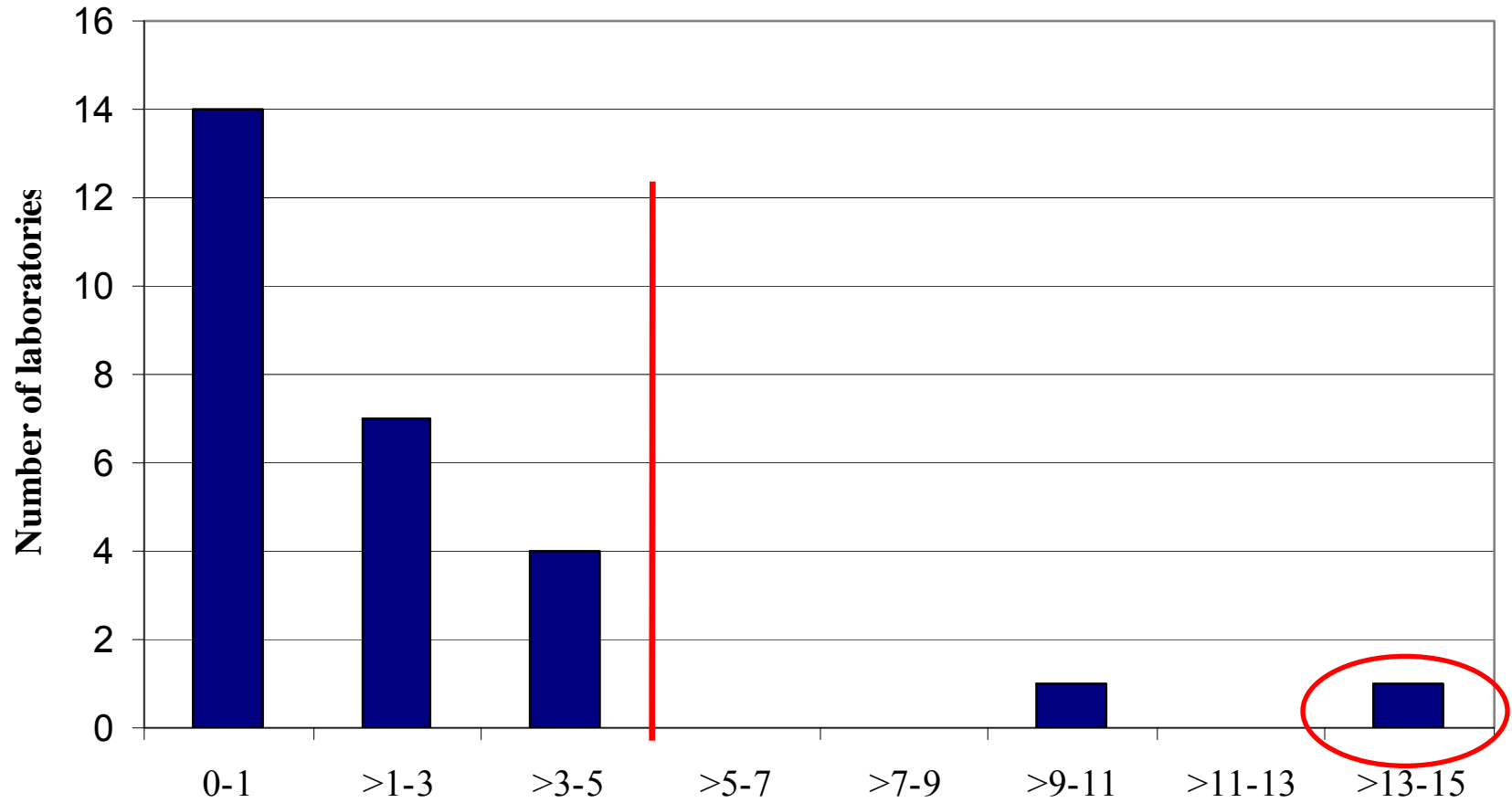
Staphylococci trial - results

- Deviation by laboratory



*Laboratories performing DD for AST

Staphylococci trial - results



25 labs

Interval of deviation

2 labs



Methicillin Resistant *S. aureus* (MRSA)

- ST.3,1, ST.3,3 and ST.3,5 were confirmed to be methicillin resistant
- 4.3% deviation
 - participant did not perform the test
 - participant did not confirm ST.3,1 but confirmed the other two MRSA strains

QC strain - *S. aureus* ATCC 25923 by DD

Antimicrobial	Deviation/Total no. of test	QC range	Min value	Max value
Cefoxitin	2/10	23-29	26	31
Chloramphenicol	0/9	16-26	18	26
Ciprofloxacin	0/10	22-30	23	29
Erythromycin	0/10	22-30	22	28.5
Florfenicol	0/7	None	20	29
Gentamicin	1/10	19-27	19	34
Penicillin	0/10	26-37	30	37
Sulfisoxazole	0/10	24-30	24.5	26

A total of 82 correct tests performed in this strain out of 85

S. aureus ATCC 25913 by MIC

Antimicrobial	Deviation/Total no. of test	QC range	Min value	Max value
Cefoxitin	0/7	1-4	2	4
Chloramphenicol	0/13	2-8	4	8
Ciprofloxacin	1/13	0.12-0.5	0.12	1
Erythromycin	0/14	0.25-1	0.25	1
Florfenicol	0/6	2-8	4	8
Gentamicin	0/13	0.12-1	0.25	≤2
Penicillin	1/12	0.25-2	0.125	2
Sulfisoxazole	0/4	32-128	32	128
Tetracycline	1/14	0.12-1	0.5	8
Trimethoprim	1/12	1-4	0.5	4

Total number of test was 108 of which 4 were incorrect

Summarizing staphylococci trial

- participants performing disk diffusion reported significantly better results than those performing MIC
- All of the strains and antimicrobials tested presented deviations below 5%.
 - The antimicrobial with the higher deviation was sulfamethoxazole (4.1%), bacteriostatic
- only two laboratories clustered outside the 5%
- Deviations in identification of the *mecA* gene were low (4.3%), and caused by two laboratories, one didn't performed the test
- Both, laboratories performing DD on *S. aureus* ATCC 25923 and laboratories using MIC on *S. aureus* ATCC 25913 produced the same percentage of deviation (4%)

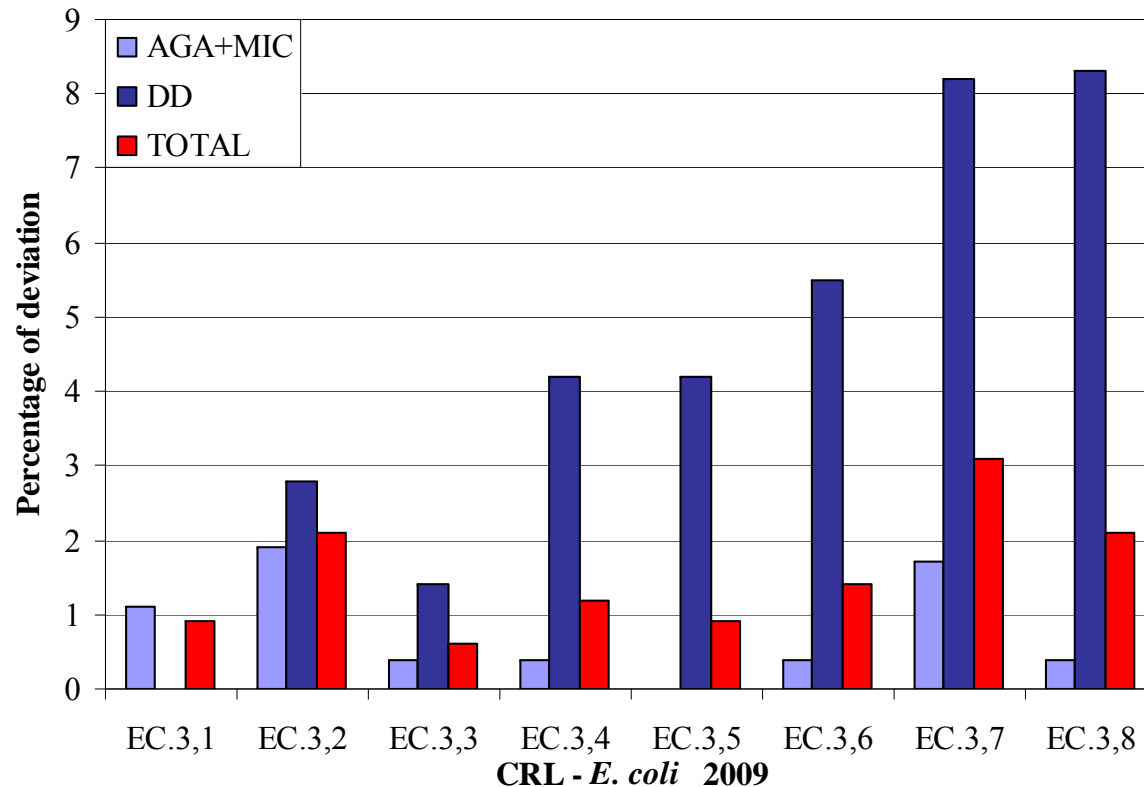
E. coli trial - results

- Results that have been omitted from the evaluation

Strain	Antimicrobial	Correct R/S	Percentage correct results	Expected MIC	Cut off value (R >)	Deviations MIC/n ¹	Deviations DD/n ²
EC.3,7	Chloramphenicol	S	71%	16	16	6/22	2/6
EC.3,7	Florfenicol	S	64%	16	16	7/21	2/4
EC.3,7	Gentamicin	S	54%	2	2	13/22	0/6
EC.3,7	Streptomycin	S	58%	8	16	9/22	2/4

E. coli trial - results

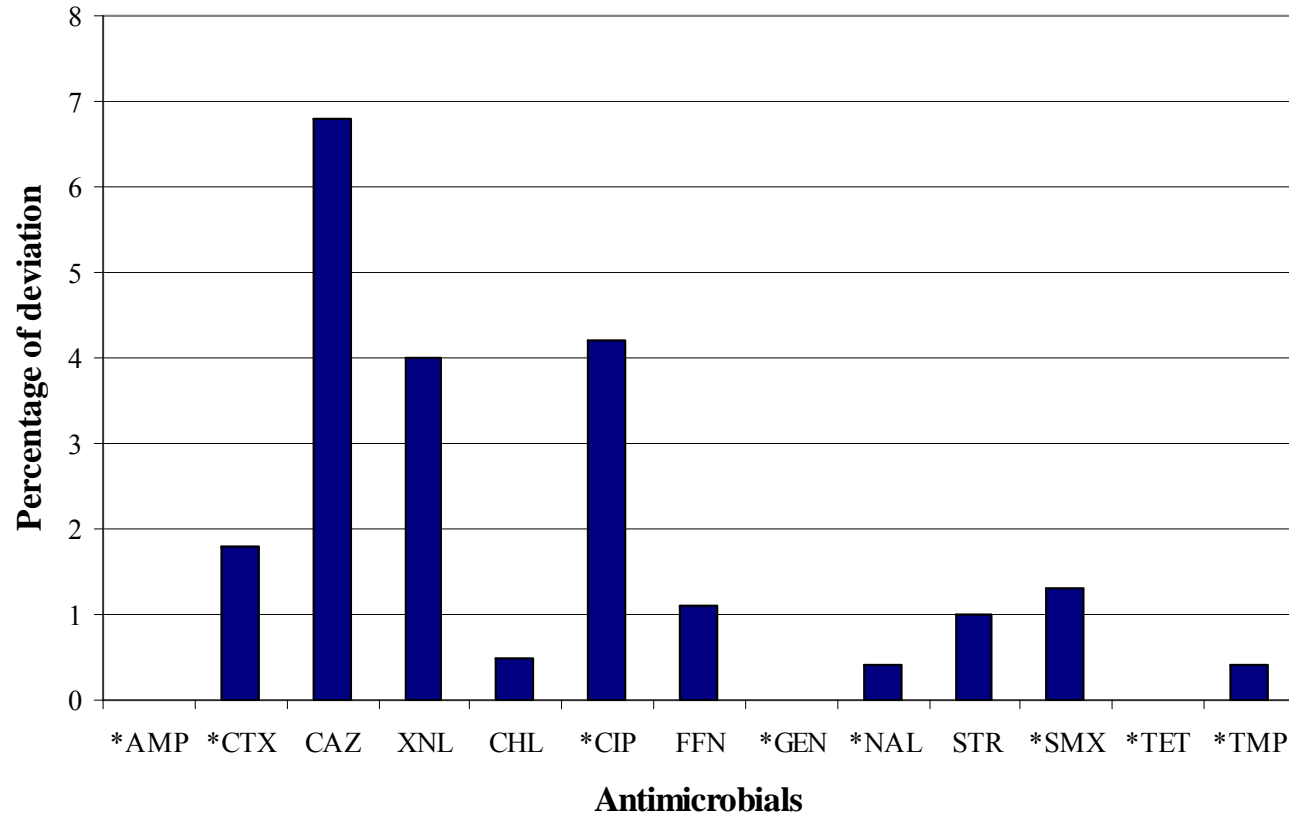
- Deviation by strain and AST method



Significant difference observed depending of method used for AST ($p < 0.01$)

E. coli trial - results

- Deviation by antimicrobial tested



*Antimicrobials recommended by EFSA for monitoring antimicrobial resistance across the EU

Ciprofloxacin resistance

- Strains EC.3,4, EC.3,6 and EC.3,8 exhibiting low-level resistance to ciprofloxacin were reported as susceptible by some participants performing DD for AST
- Discrepancy on the cut off values recommended by EFSA ($>0,032$ mg/L) and those recommended by CLSI (≥ 4 mg/L) for the MIC interpretation of ciprofloxacin.
- For those participants performing disk diffusion, the EURL-AR recommends the use of a low concentration of ciprofloxacin (1 μ g) in the disks as it appeared to increase the sensitivity of the assay

Cephalosporin resistant strains

EC.3,2	<i>bla</i> _{TEM52}
EC.3,5	<i>bla</i> _{CTX-M-1}
EC.3,7	<i>bla</i> _{CTX-M-9}
EC.3,8	<i>bla</i> _{CMY-2}

- 5/28 labs failed to identify ESBL producing organisms

-#40 obtained susceptible results for all cephalosporins tested

- #23 and #39 did not perform any of the confirmatory tests

-#29 obtained synergy for EC. 3,7 in the confirmatory test (CAZ:CAZ/CL) and failed to interpret correctly

-#20 did not obtain synergy in any of the two confirmatory tests for EC.3,7 (CAZ:CAZ/CL and CTX:CTX/CL) but results for ceftiofur, cefotaxime and ceftiofur were correct.

AmpC strain

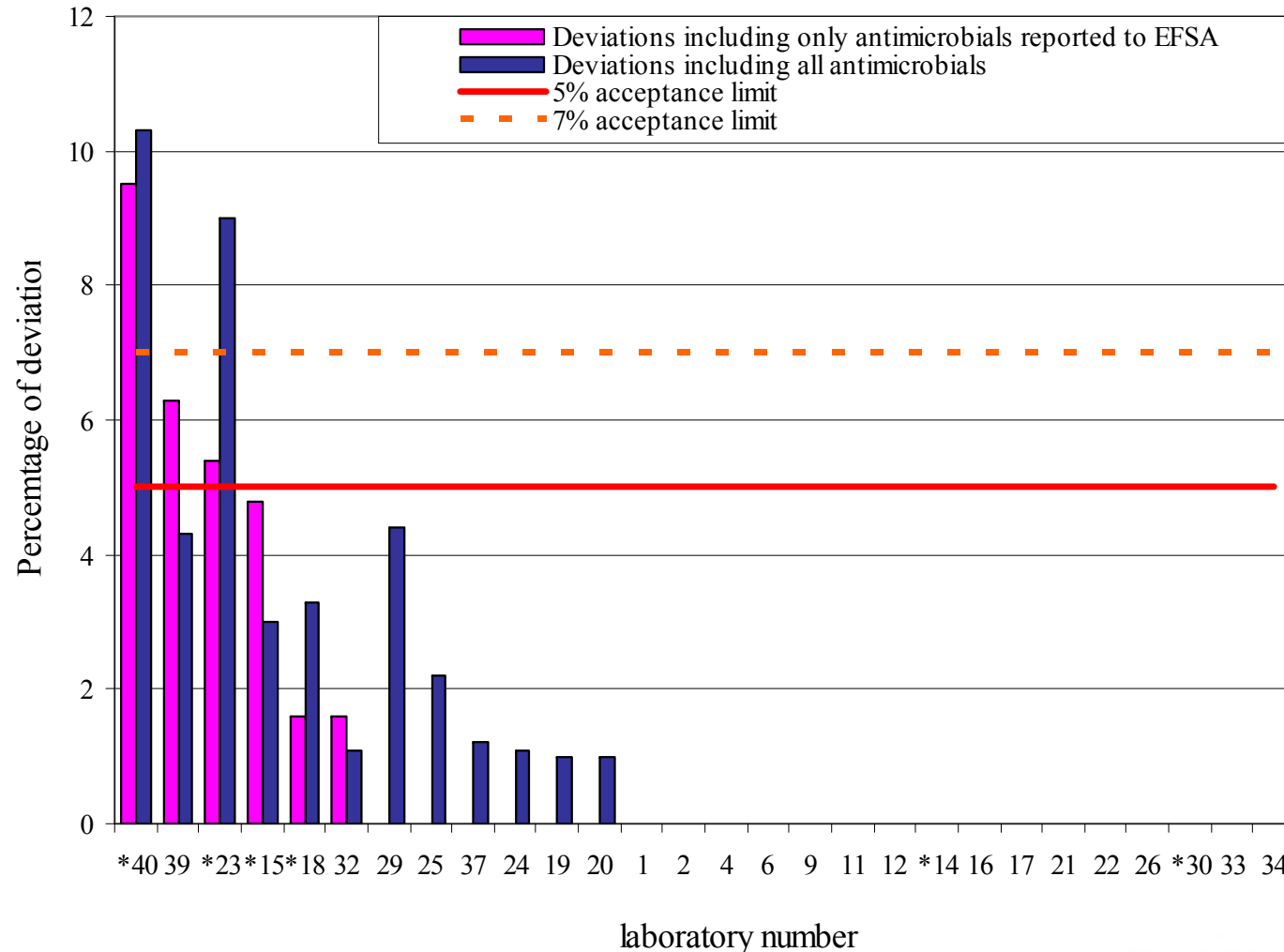
- 7/28 labs failed to identify the ampC strain EC.3,8
 - #23 obtained susceptible values for all cephalosporins tested
 - #4 identified the strain as ESBL. Both confirmatory test were positive
 - #22 and #37 identified the strain as ESBL and ampC
 - #24 and #29 obtained resistance values for cephalosporins but did not performed confirmatory test or AST for cefoxitin
 - #30 performed all tests and got correct results even for cefoxitin but fail to interpret them correctly

“concerning cefotaxime, ceftazidime and/or ceftiofur used when detecting ESBL-producing strains in the EQAS: If a microorganism is resistant to one or two of these drugs, it should be regarded resistant to all three (this does not include cefoxitin, as ampC’s are resistant to cefoxitin and ‘true ESBLs’ are not).“



E. coli trial - results

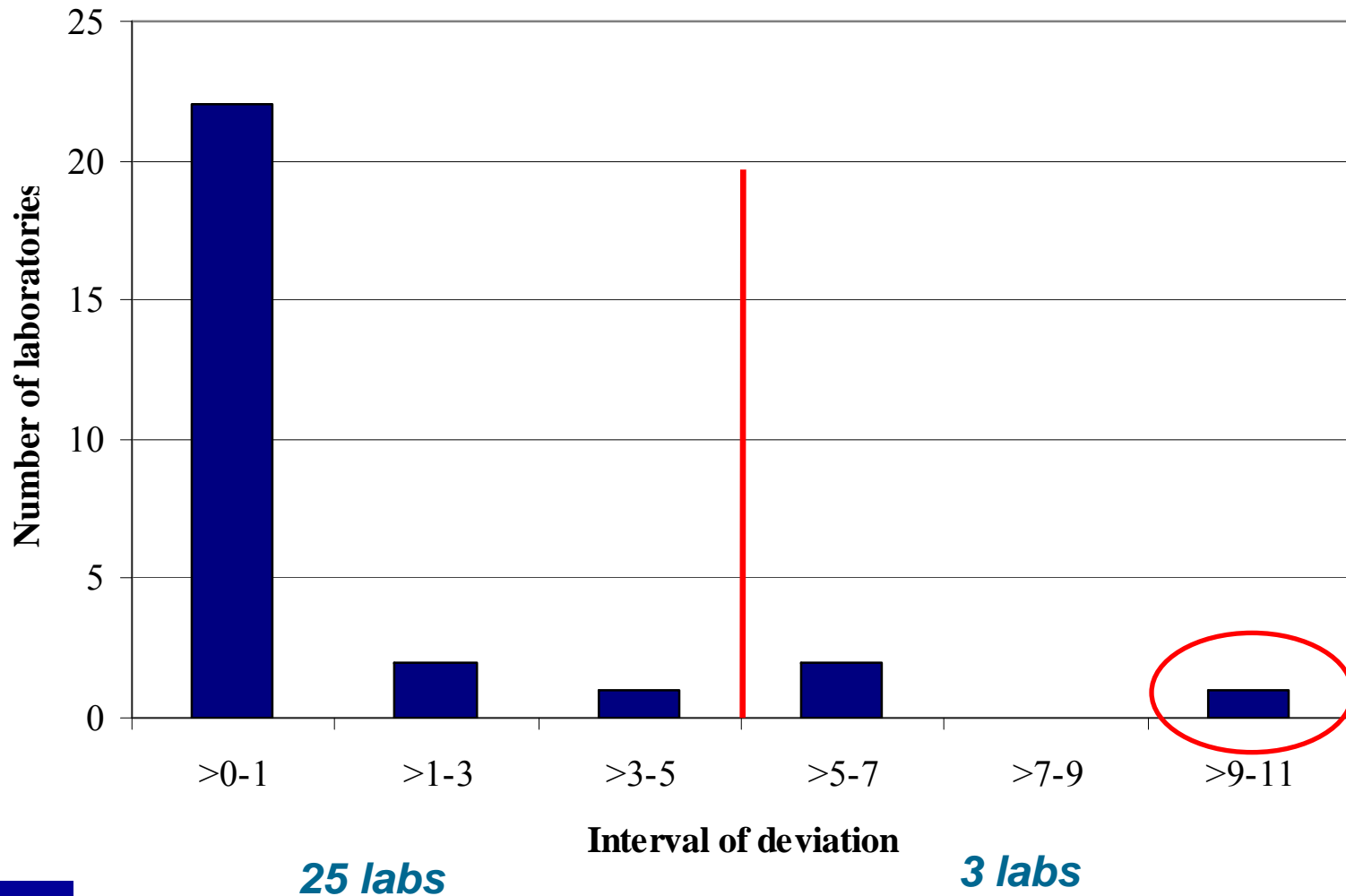
- Deviation by laboratory



*Laboratories performing DD for AST



E. coli trial - results



QC strain - *E. coli* ATCC 25922 by DD

-48 tests only 1 incorrect

-Decrease in the deviation from 12.3% in 2008 to 2% this year

Antimicrobial	Deviation/Total no of test	QC range	Min value	Max value
Cefotaxime, CTX	0/3	16-22	32	32
Ceftazidime, CAZ	0/4	29-35	27	29
Ceftiofur, XNL	1/5	26-31	24	28
Chloramphenicol, CHL	0/5	21-27	22	26.3
Ciprofloxacin, CIP	0/4	30-40	34	40
Florfenicol, FFN	0/2	22-28	23	27
Gentamicin, GEN	0/5	19-26	20	24.4
Nalidixic acid, NAL	0/5	22-28	25	27
Sulfisoxazole, FIS	0/5	15-23	18	23
Tetracycline, TET	0/5	18-25	20	25
Trimethoprim, TMP	0/5	21-28	21	26

QC strain - *E. coli* ATCC 25922 by MIC

- 237 test performed of which 4 were incorrect (deviation 1.7%)

Antimicrobial	Deviation/Total no of test	QC range	Min value	Max value
Ampicillin, AMP	1/21	2-8	1	8
Cefotaxime, CTX	0/21	0.03-0.12	0.06	0.12
Ceftazidime, CAZ	0/13	0.06-0.5	0.12	0.25
Ceftiofur, XNL	0/6	0.25-1	0.25	0.5
Chloramphenicol, CHL	0/21	2-8	4	8
Ciprofloxacin, CIP	2/21	0.004-0.016	0.008	0.03
Florfenicol, FFN	0/19	2-8	4	8
Gentamicin, GEN	0/21	0.25-1	0.25	1
Nalidixic acid, NAL	0/21	1-4	1	4
Streptomycin, STR	0/19	4-16	4	8
Sulfisoxazole, FIS	1/14	8-32	16	64
Tetracycline, TET	0/21	0.5-2	1	2
Trimethoprim, TMP	0/19	0.5-2	0.5	1

Summarizing *E. coli* trial

- The best results for *E. coli* when compared to previous EQAS. Decrease in the average deviation for the *E. coli* trial, from 2.1% from EQAS 2008 to 1.5%
- Deviations were mainly caused by laboratories performing DD for AST
- Only three NRLs exhibited deviations higher than 5% :
 - identification of low level ciprofloxacin resistance
 - cephalosporin resistance
- Deviations for ESBL and ampC detection are still high
- For *E. coli* ATCC 25922, the percentage of positive results for all test performed has increased from 90% in EQAS 2007 to 96.8% in 2008 and 98.3% this year

Conclusions

- Performance has improved in the three trials
- Enterococci needs attention regarding the antimicrobials recommended by EFSA (aminoglycosides)
- MRSA identification has improved
- Ciprofloxacin resistance harmonization of cut off values
- ESBL producing *E. coli* still considered a priority area
- Main cause of deviations
 - Strains with expected MIC values close to the epidemiological cut off values to define them as resistant
 - Laboratories performing disk diffusion
- No outliers have been identified this year, however, laboratories with high percentage of deviations would be contacted

Thank you for your attention

