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EQAS 2008

Enterococci, Staphylococci *and E. coli*

CRL workshop, April 23, 2009

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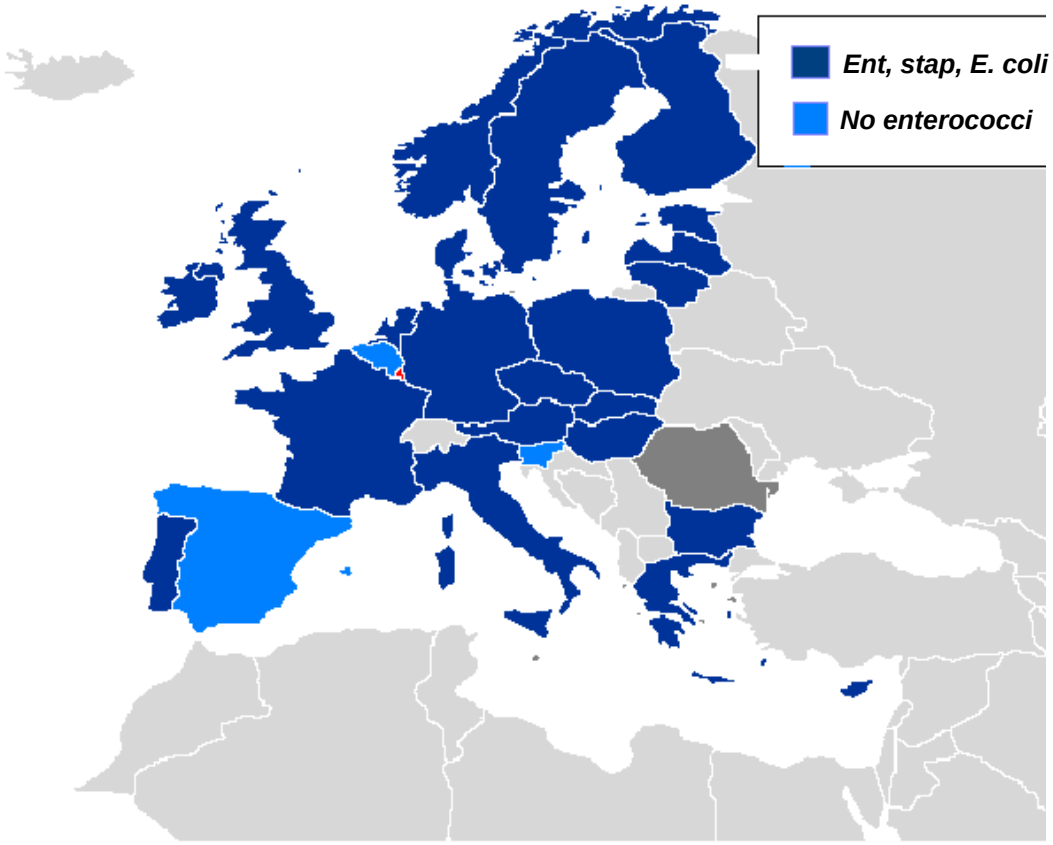
Main objectives of the CRL EQAS's

- To improve the comparability of antimicrobial susceptibility testing (AST) data
- To harmonise the breakpoints/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, 2008



Number of participating labs

	2007	2008
Enterococci	26	23
Staphylococci	31	28
E. coli	30	27

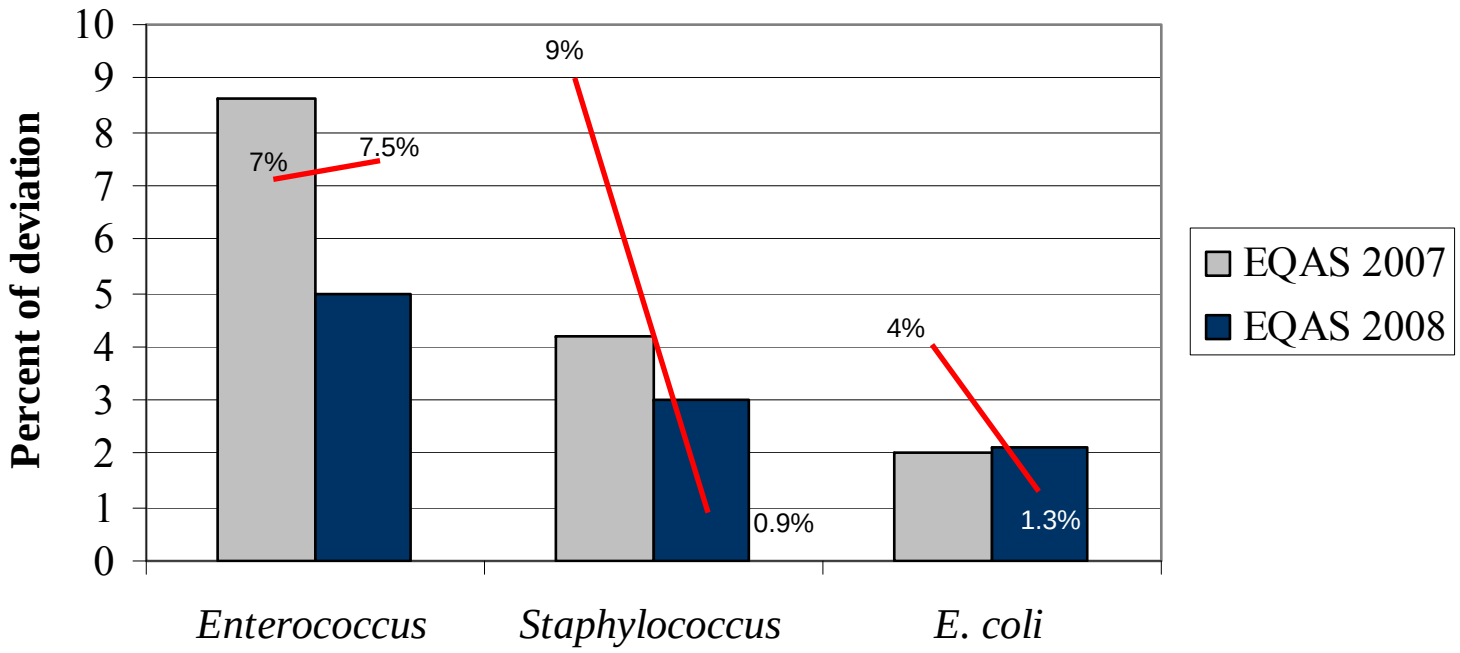
Methods for EQAS 2008

- 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 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 sensitive

Analysis of data based on these agreements

- During the passed CRL-AR Workshop (2008) the network agreed upon the following decisions for EQAS 2008:
  - The accepted deviation for each laboratory was set up at 7%
  - Results should be further analysed (possibly ignored) if only 75% are correct (test strain/antimicrobial combination)
  - harmonising AST analyses by MIC determination using the antimicrobial panel and cut-off values recommended by EFSA

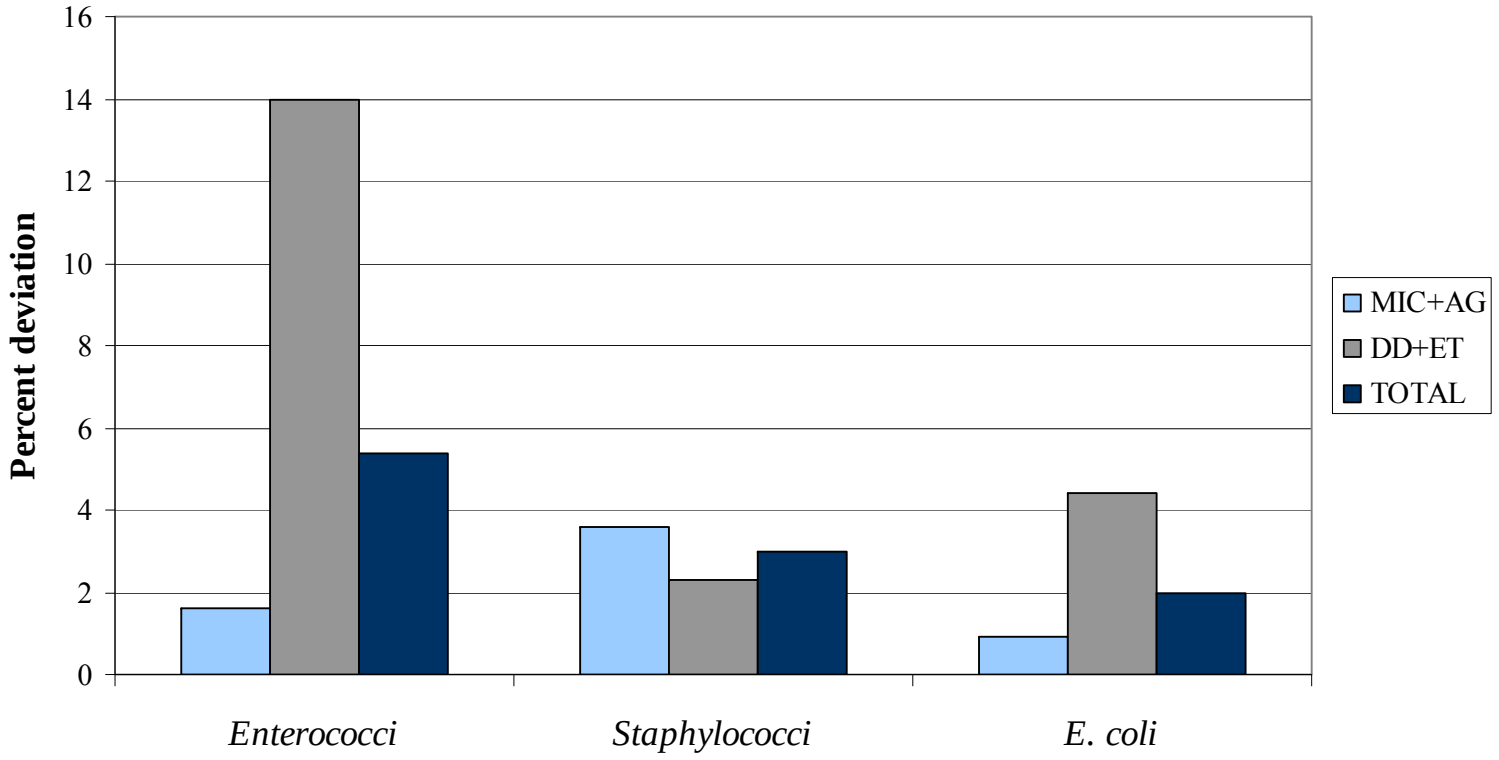
EQAS 2008 versus EQAS 2007



Percentage of positive results

	2007	2008
Enterococci	91.4%	95%
Staphylococci	95.8%	96.9%
E. coli	98%	97.9%

Deviation by strain comparing the AST methods



- Significant differences observed for enterococci and *E. coli* depending on the AST method ($p < 0.01$)

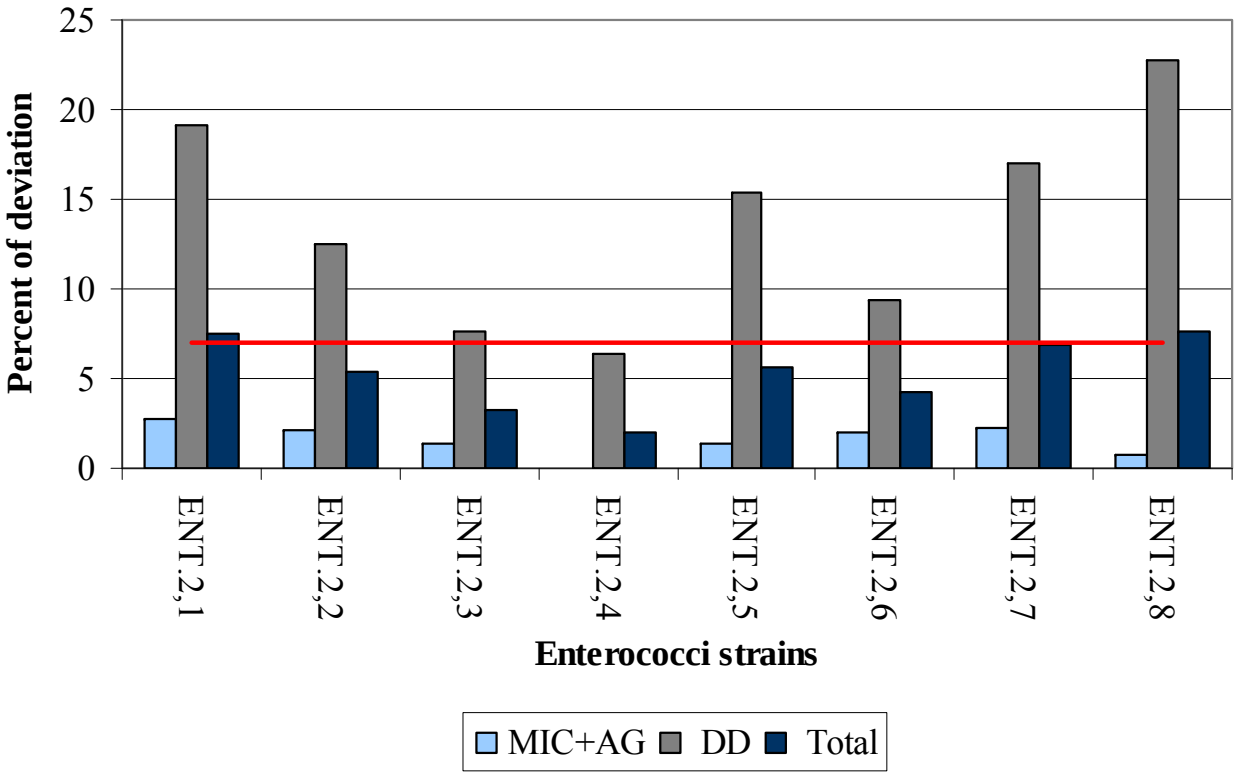
Enterococci trial - results

- 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.2,2	Synacid	S	**63%**	16	32	2/7	1/1
ENT.2,4	Ampicillin	S	**45%**	4	4	9/15	3/7
ENT.2,4	Ciprofloxacin	S	**67%**	4	4	1/4	4/5
ENT.2,4	Streptomycin	R	**25%**	256	128	13/14	2/6
ENT.2,7	Daptomycin	S	**67%**	4	4	1/3	0
ENT.2,7	Synacid	S	**44%**	1	1	5/8	0/1
ENT.2,8	Daptomycin	S	**33%**	4	4	2/3	0

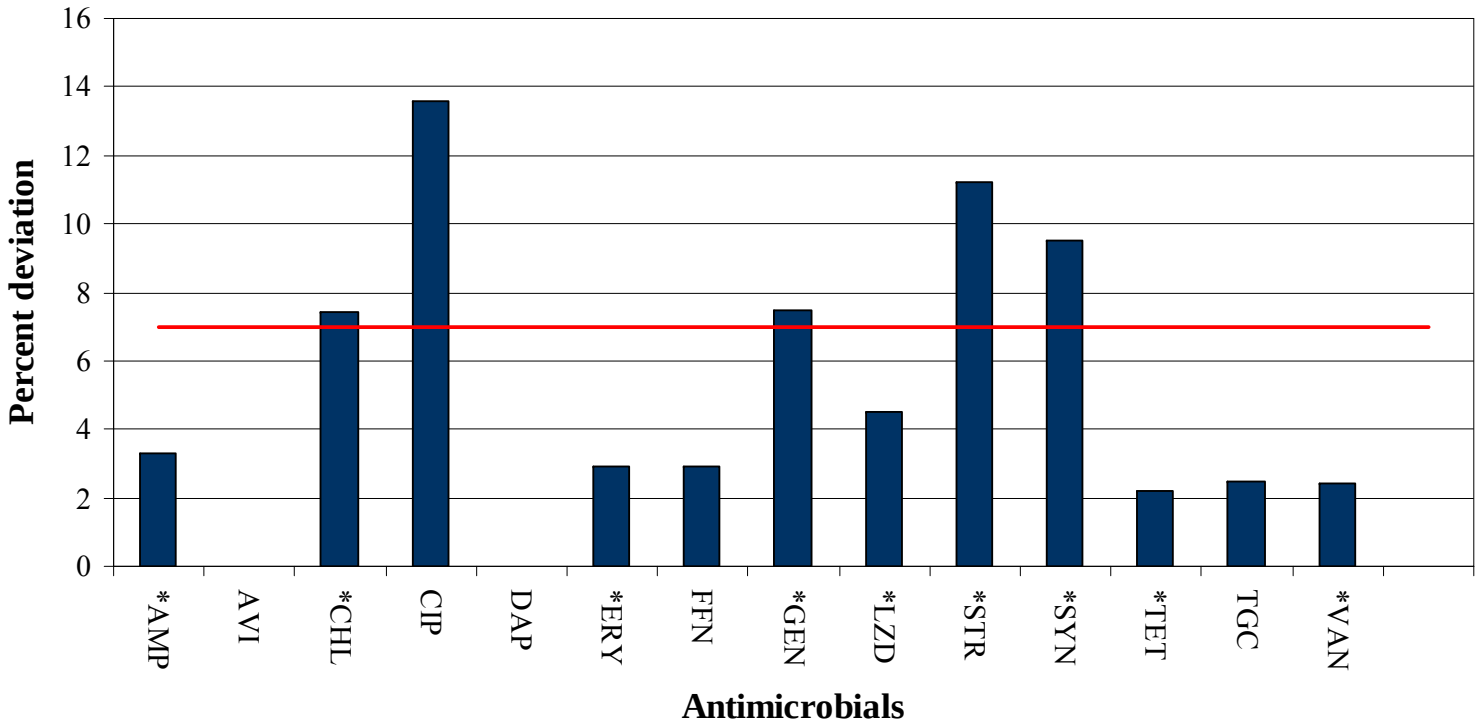
Enterococci trial - results

- Deviation by strain and AST method



Enterococci trial - results

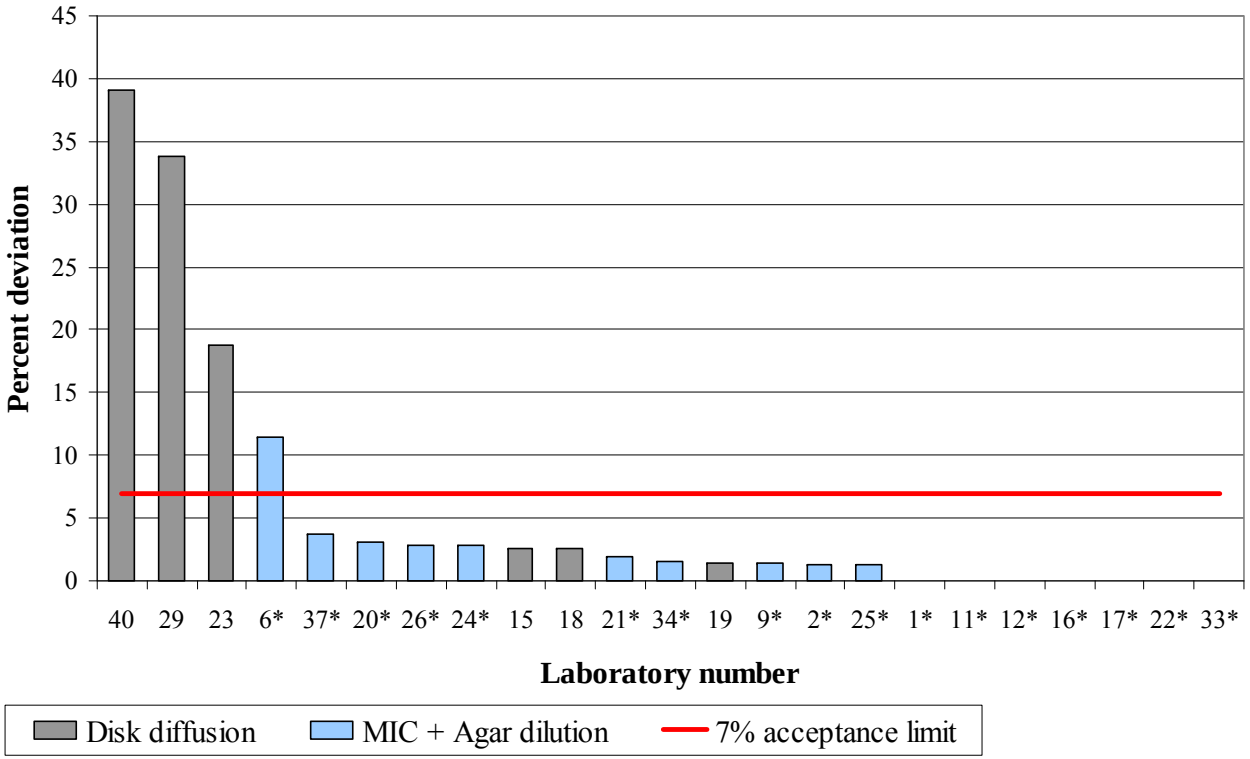
- Deviation by antimicrobial tested



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

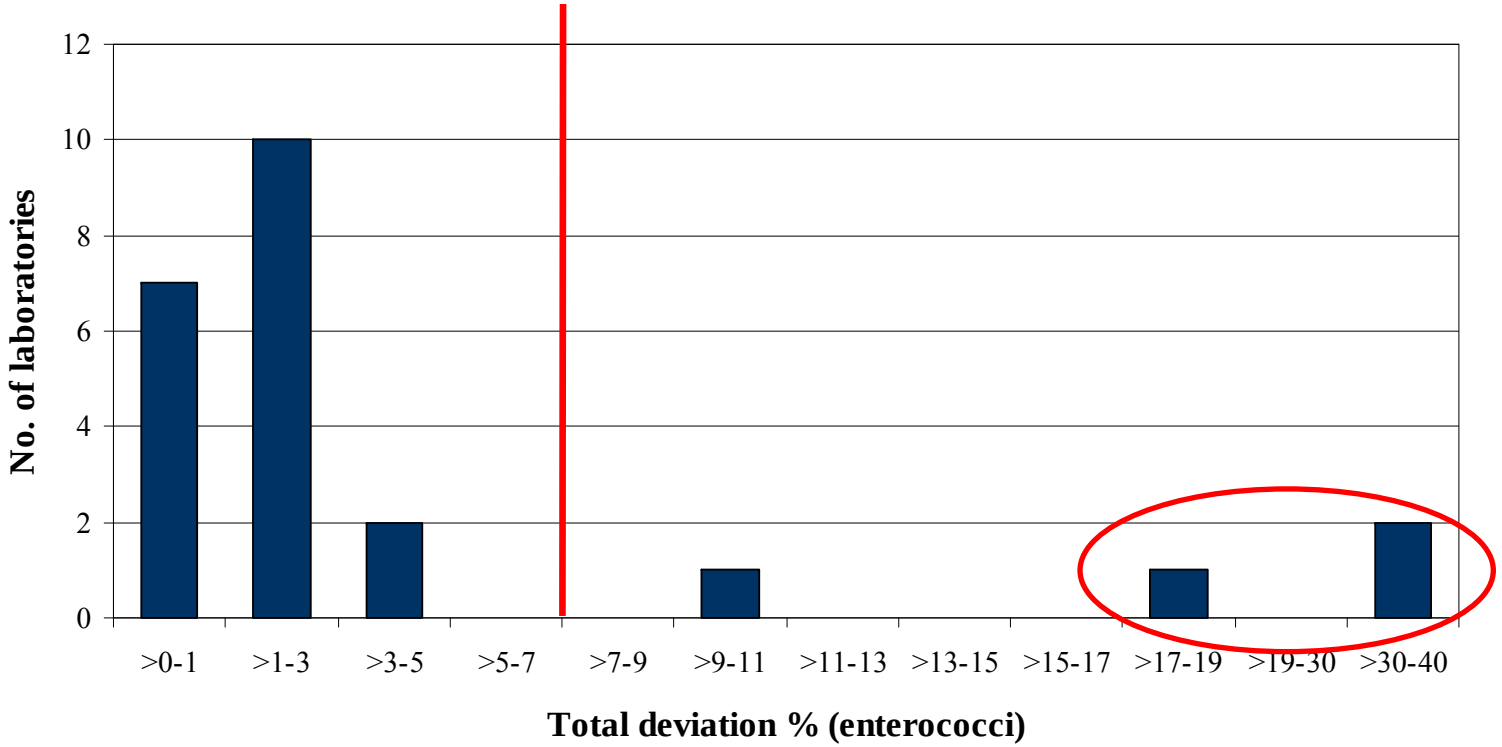
Enterococci trial - results

- Deviation by laboratory



*Laboratories performing MIC for AST

Enterococci trial - results



19 labs

4 labs

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QC-STRAIN MIC

127 correct tests performed in this strain

E. faecalis ATCC 29212				
Antimicrobial	**MIC deviations /Total no. of test**	**QC range MIC**	**Min value**	**Max value**
Ampicillin	**0**/15	0.5 - 2	0.5	2
Avilamycin	**0**/3	0.5 - 4	01	4
Chloramphenicol	**0**/15	4 - 16	4	8
Ciprofloxacin	**0**/9	0.25 - 2	0.5	1
Daptomycin	**0**/3	1 - 8	1	2
Erythromycin	**0**/14	1 - 4	1	4
Florfenicol	**0**/6	2 - 8	2	4
Gentamicin	**0**/14	4 - 16	8	≤128
Linezolid	**0**/10	1 - 4	1	2
Synacid	**0**/7	2 - 8	4	8
Tetracycline	**0**/15	8 - 32	16	32
Tigecycline	**0**/3	0.03 – 0.12	0.06	0.12
Vancomycin	**0**/13	1 - 4	1	4

Summarizing enterococci trial

- 4/9 antimicrobials recommended by EFSA failed to produce 100% of correct results
- Only 5 antimicrobials have deviated in this EQAS 2008 by comparison to the 7 that deviated in EQAS 2007
- The number of laboratories deviating more than the 7% acceptance limit has decreased, from 14 in 2007 to 4, with the majority clustered in the deviation interval between 0% and 3%
- Deviations were mainly caused by laboratories performing DD for AST
- three laboratories identified as outliers
- MIC for QC *E. faecalis* ATCC 29212 revealed no deviation (EQAS 2007 deviation was 1.8%)

Staphylococci trial - results

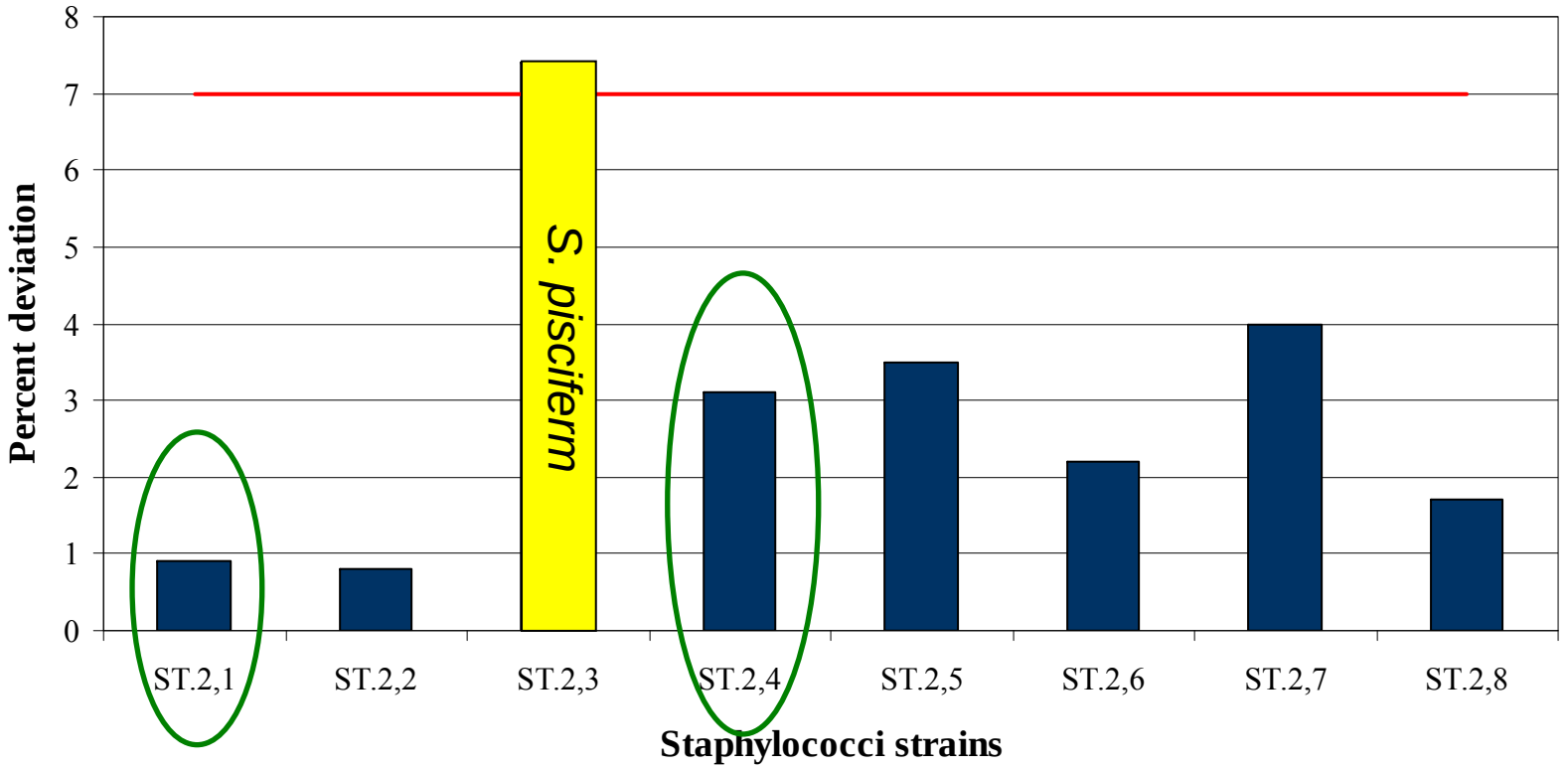
- Results that have been omitted from the evaluation

Strain	Antimicrobial	Correct R/S	Percentage correct results	Expected MIC	Cut off value (R >)	Deviations MIC/n[1]	Deviations DD/n[2]
ST.2,1	Ciprofloxacin	R	**38%**	2	1	9/17	9/11
ST.2,6	Tetracycline	R	**50%**	4	1	3/17	11/11
ST.2,8	Streptomycin	S	**36%**	16	32	6/12	8/10

- Tetracycline pH dependent (?)

Staphycocci trial - results

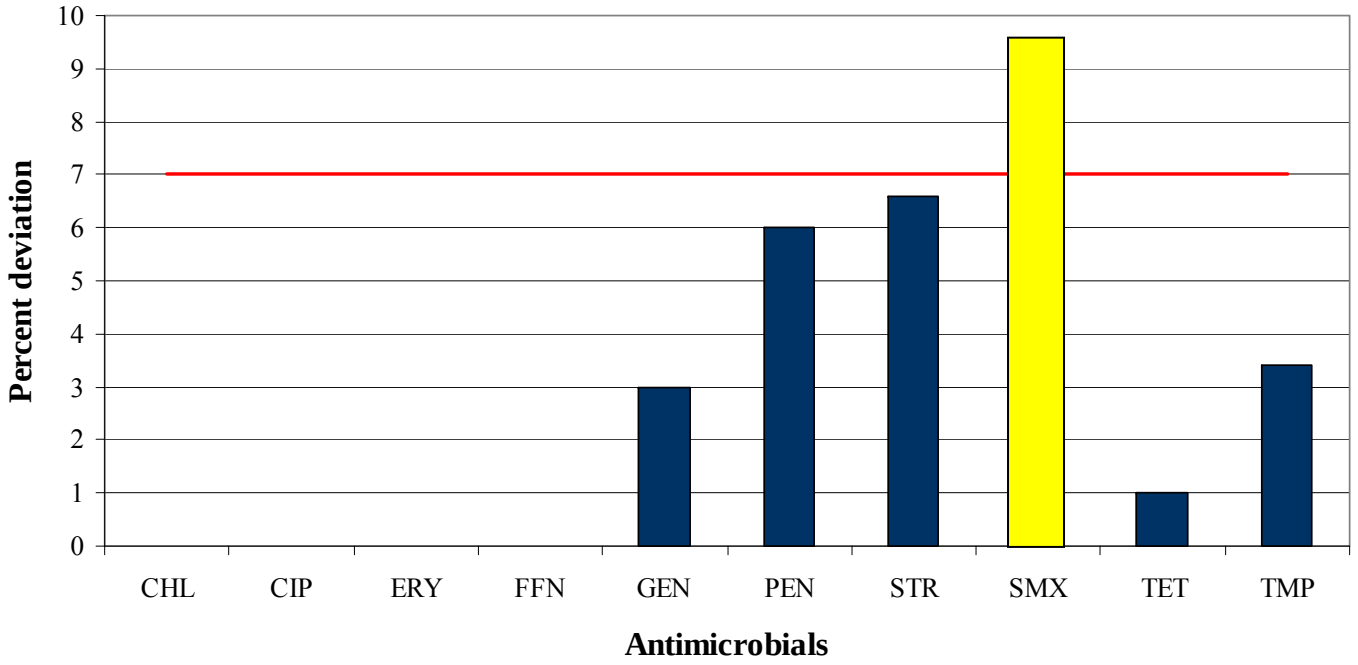
- Deviation by strain



8/27 labs failed to identify *mec*A in one or more test, 30% of the labs by comparison to the 17% that failed in 2007

Staphylococci trial - results

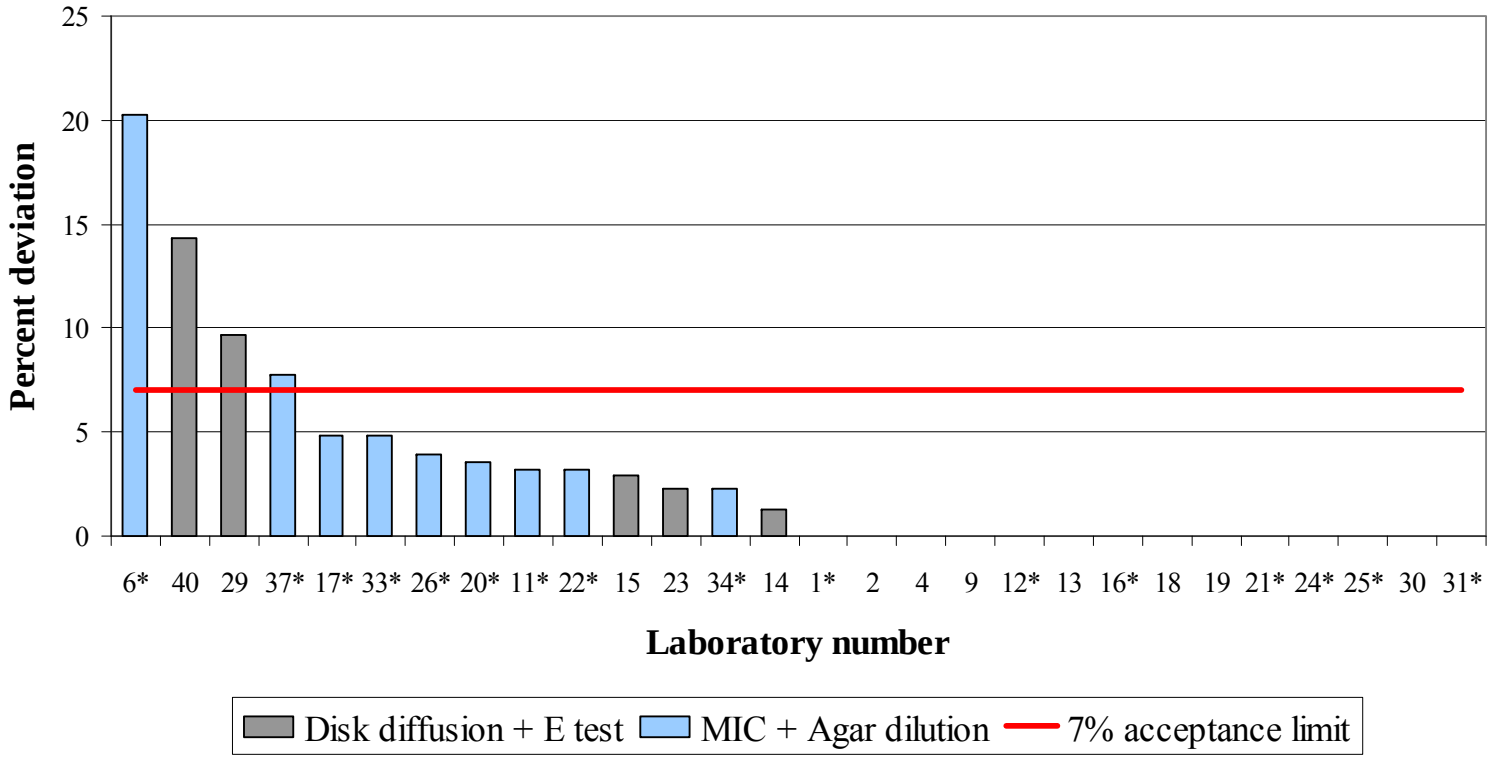
- Deviation by antimicrobial tested



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

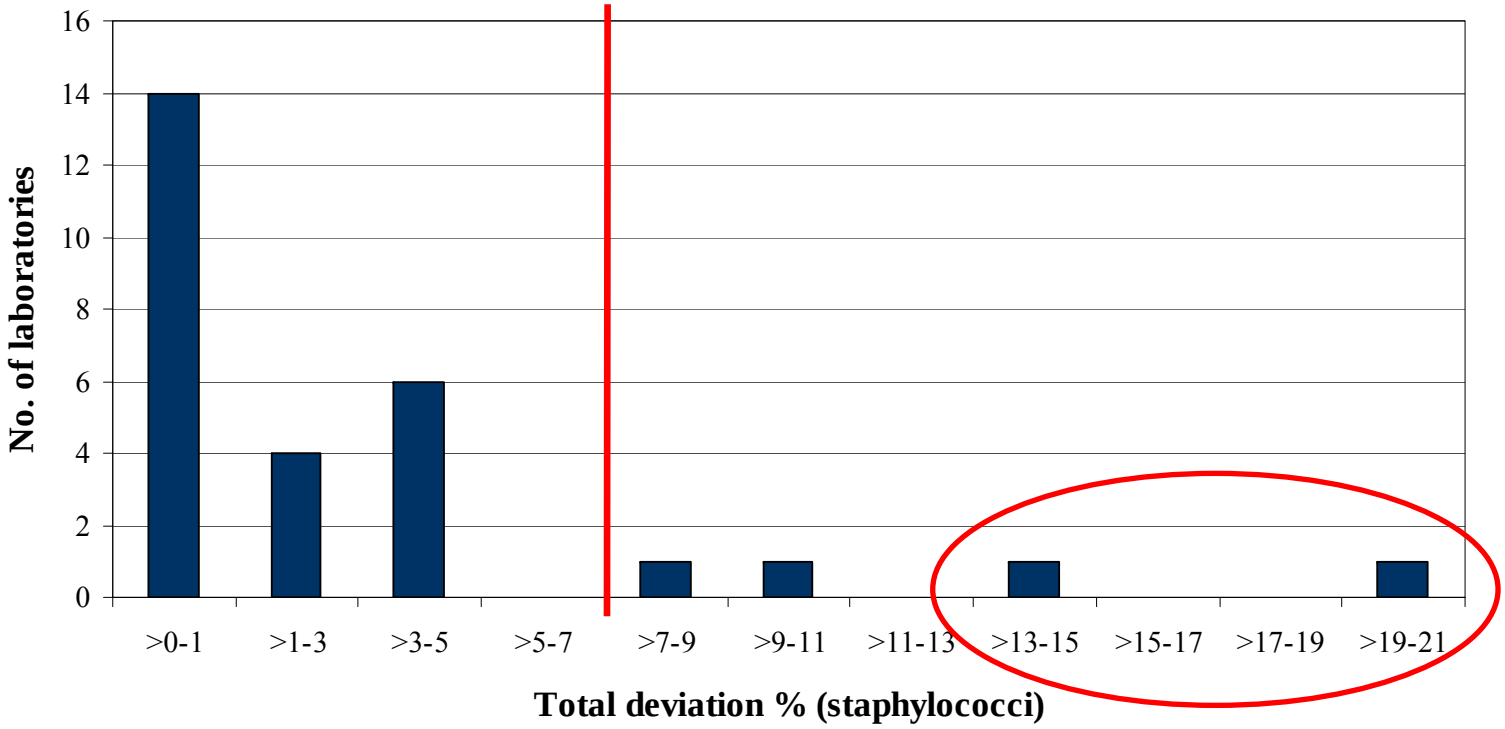
Staphylococci trial - results

- Deviation by laboratory



*Laboratories performing MIC for AST

Staphylococci trial - results



24 labs

4 labs

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

Antimicrobial	QC range	Deviation/Total no. of test	Min value	Max value
Chloramphenicol	16 - 26	0/9	18	24
Ciprofloxacin	22 - 30	0/11	22	30
Erythromycin	22 - 30	**2/11 (18.2%)**	20	31
Gentamicin	19 - 27	**1/11 (9.1%)**	19	29
Penicillin	26 - 37	**1/11 (9.1%)**	30	40
Streptomycin	14 - 22	**1/9 (11.1%)**	14	31
Sulfisoxazole	24 - 30	**2/7 (28.6%)**	6	26
Tetracycline	24 - 34	0/11	24	30
Trimethoprim	19 - 26	**1/8 (12.5%)**	16	24

Total number of test was 96, of which 8 were incorrect producing a deviation of 8,3%

S. aureus ATCC 25913 by MIC

Antimicrobial	QC range	Deviation/Total no. of test	Min value	Max value
Chloramphenicol	2 - 8	0/13	4	8
Ciprofloxacin	0,12 – 0,5	0/12	0,12	≤1
Erythromycin	0,25 - 1	0/12	≤0,25	0,5
Florfenicol	2 - 8	0/8	2	4
Gentamicin	0,12 - 1	0/11	≤0,25	0,5
Penicillin	0,25 - 2	0/12	0,25	1
Streptomycin	0 - 256	0/8	≤2	≤1000
Sulfisoxazole	32 - 128	0/5	32	128
Tetracycline	0,12 - 1	0/13	0,5	4
Trimethoprim	1 - 4	0/10	1	2

A total of **104** correct tests performed in this strain

Summarizing staphylococci trial

- Two laboratories identified as outliers by comparison to the 7 from EQAS 2007
- 30% of the laboratories failed to detect MRS in one or two tests
- MICs for the QC strain *S. aureus* ATCC 25913 were 100% positive whereas in 2007 this percentage was 94.1%
- DD for *S. aureus* ATCC 25923 showed a reduction in the deviation from 18.3% in the EQAS 2007 to 8.3% in 2008
- Next year MRSA detection will be mandatory and a protocol is posted in the web

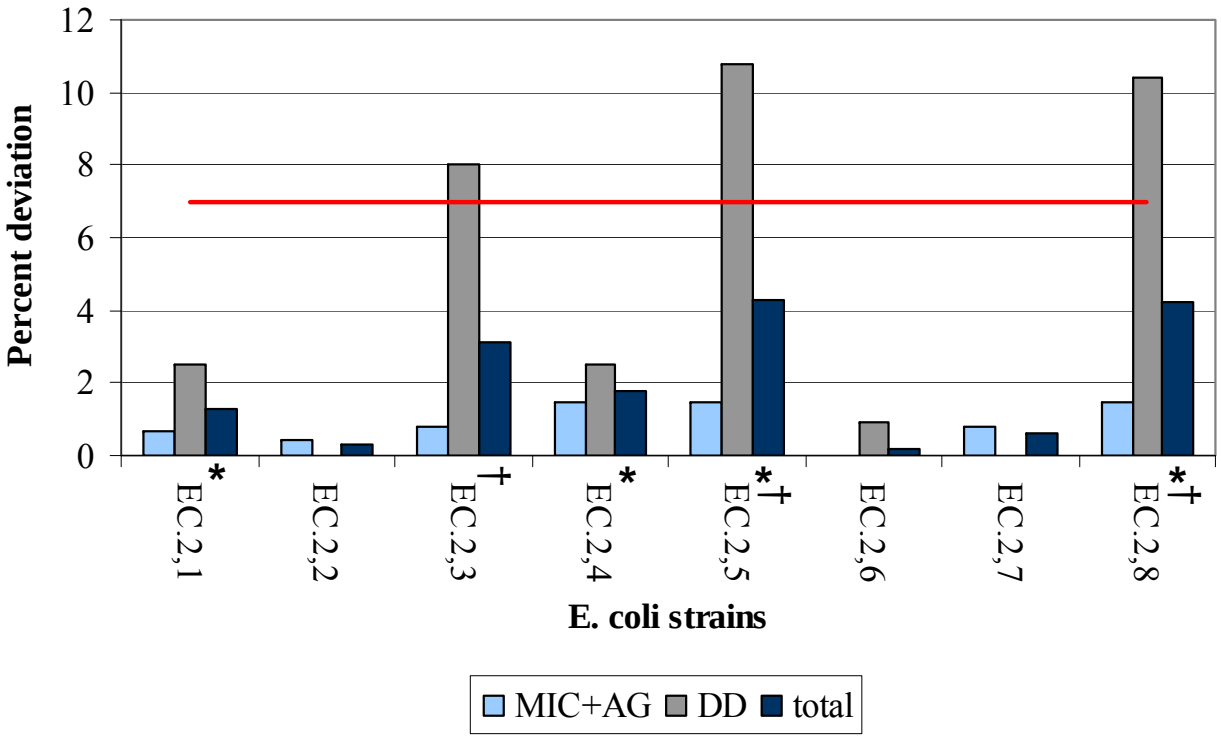
E. coli trial - results

- 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.2,2	Streptomycin	S	12%	16	16	16/19	7/8
EC.2,5	Amoxicillin + clavulanic ac	S	50%	8	8	1/2	4/8

E. coli trial - results

- Deviation by strain and AST method

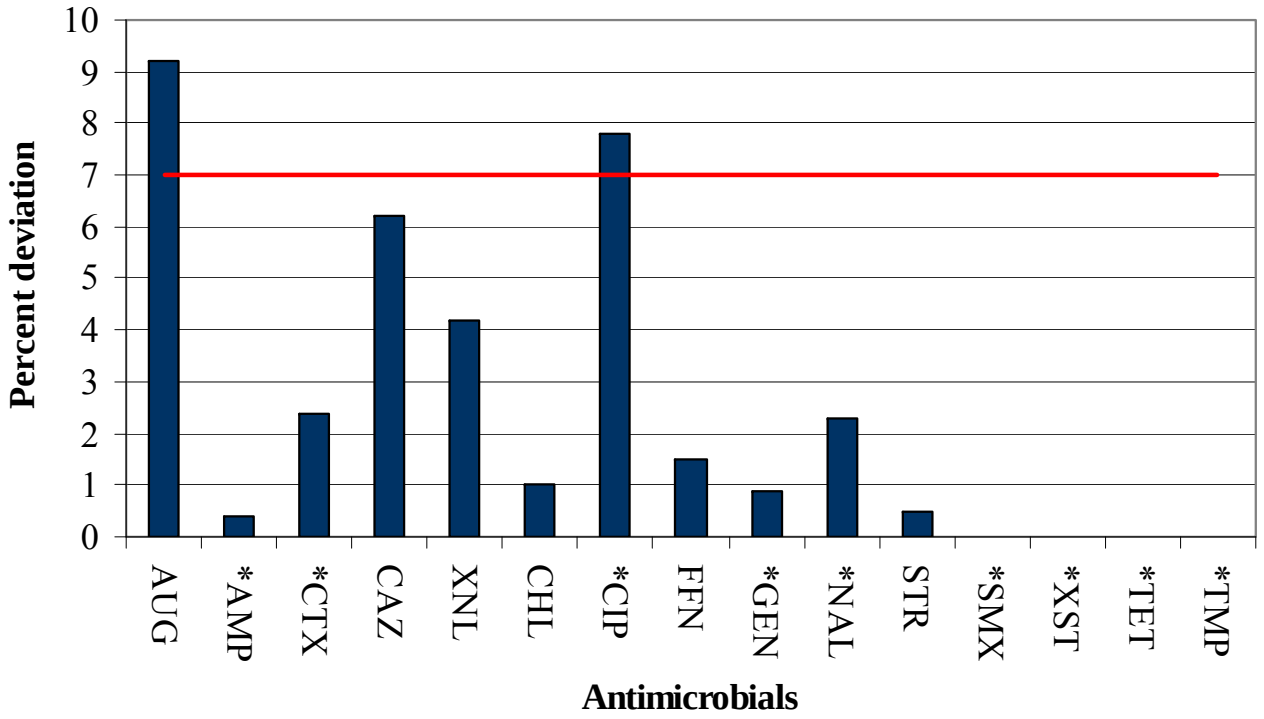


* *ESBL producing*
† Low level cip-R

Significant difference observed depending of method used for AST

E. coli trial - results

- Deviation by antimicrobial tested



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

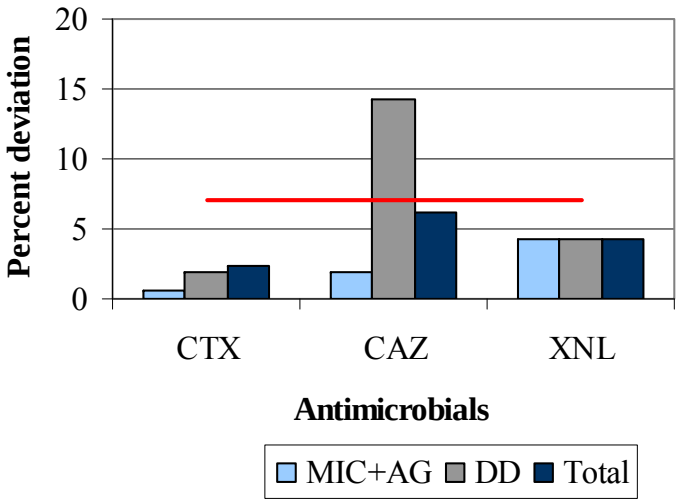
Ciprofloxacin resistance

Strain	Mutation /Gene	Correct R/S	Correct results (%)	Expected MIC	Cut off value (R>)	Deviation MIC/n[1]	Deviation DD/n[2]
EC.2,3	GyrA	R	**72%**	0.06	0.032	**1/18**	**6/7**
EC.2,5	QnrS1	R	**85%**	0.5	0.032	0/19	**4/7**
EC.2,8	QnrS2	R	**80%**	0.12	0.032	0/19	**5/6**

Laboratories performing MIC produced higher number of correct results when compared with DD which ended up causing 94% of the deviation

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.

ESBL producing strains



cefotaxime (CTX), ceftazidime (CAZ) and ceftiofur (XNL)

- 2/25 labs failed to identify ESBL producing organisms in one or two strains

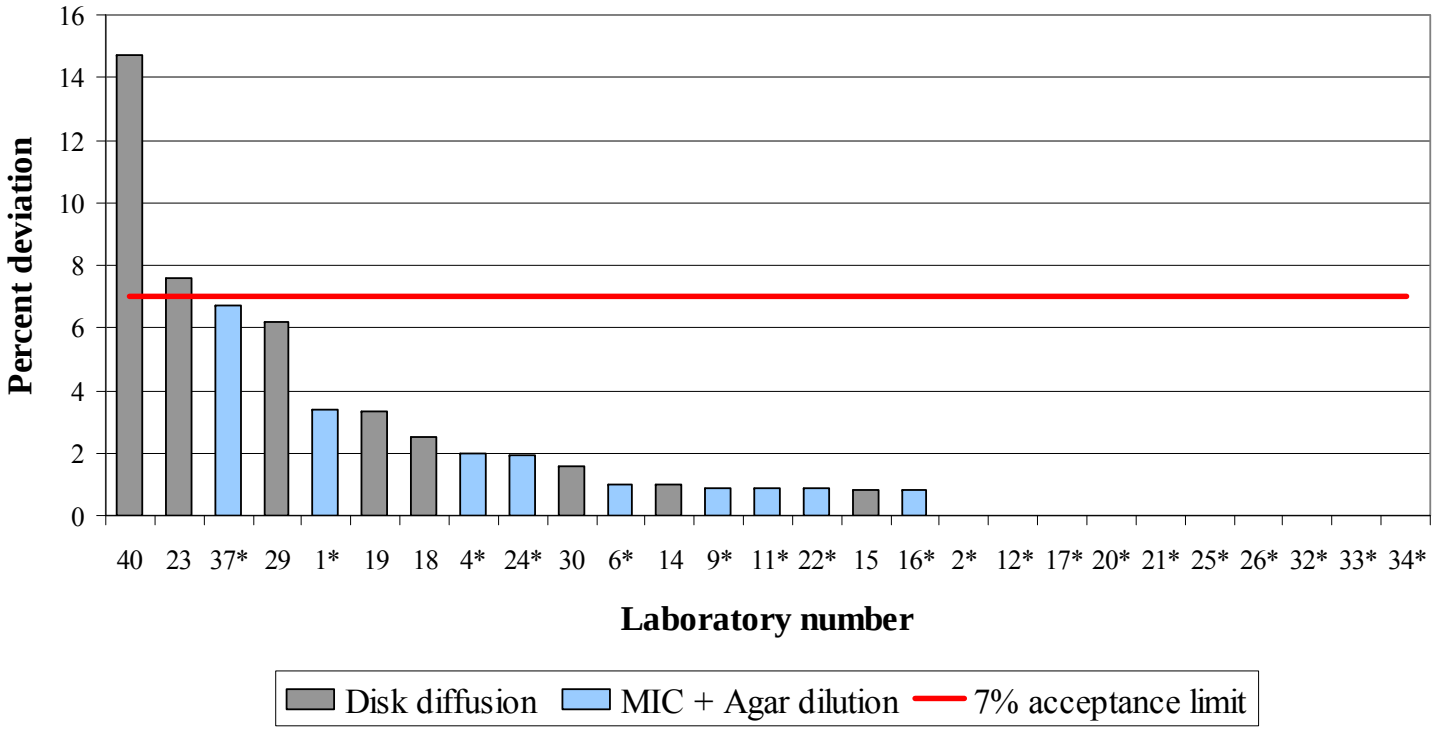
  - Lab #1 in one of the cases produced an error on the interpretation of results.

  - Labs #1 & #6, the diameter zones for the two tests were smaller than expected. Deviations caused by a methodological error

Remember: if one cephalosporin shows resistance, all cephalosporins should be regarded as resistant

E. coli trial - results

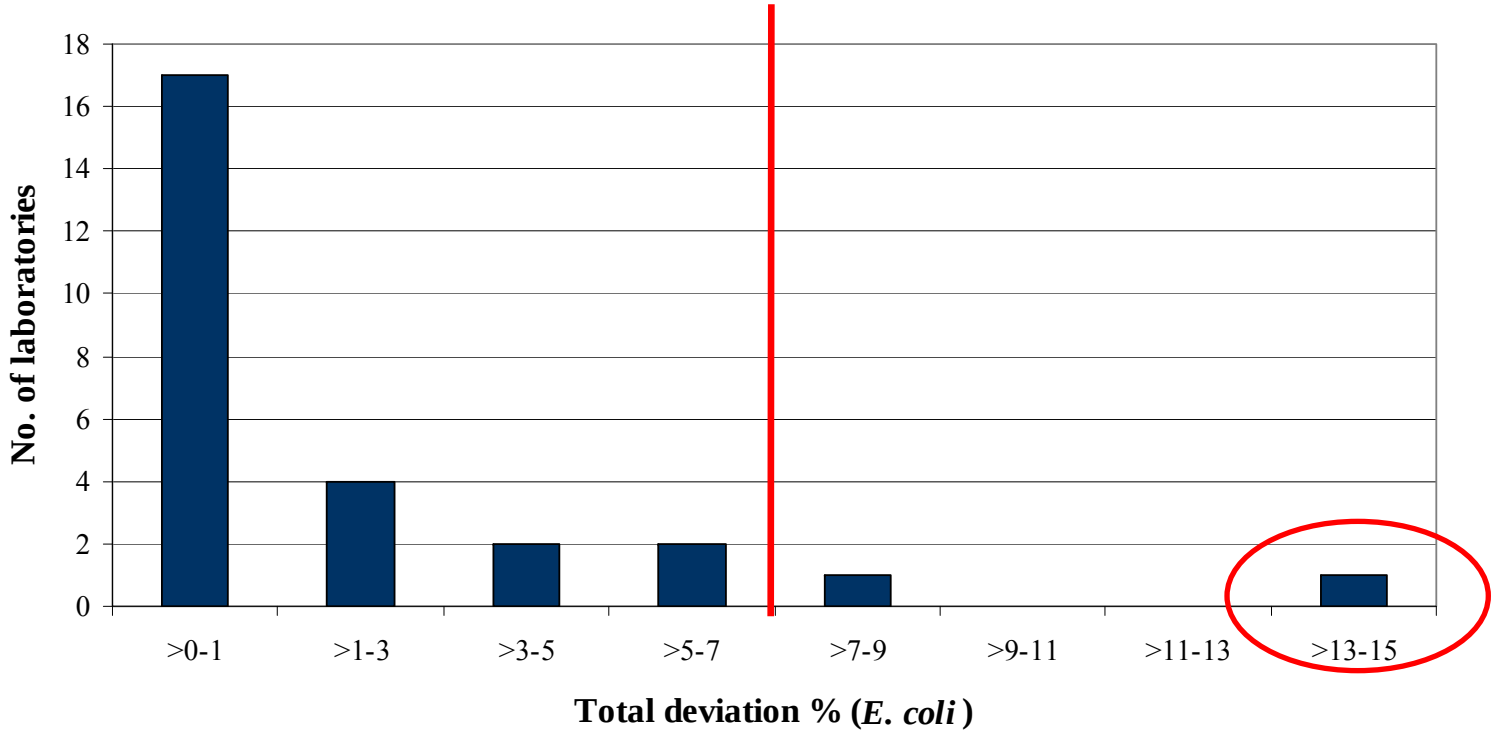
- Deviation by laboratory



*Laboratories performing MIC for AST

10 labs with 100% correct results performed MIC

E. coli trial - results



25 labs

2 labs

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

-**106** test performed of which 13 were incorrect

- 7/13 incorrect results were caused by one participant

-the deviation for this strain was 12.3%, slight increase when compared to 2007 (11.1%)

Antimicrobial	QC range	Deviation/Total no of test (%)	Min value	Max value
Amoxicillin+clavulanic ac	18 – 24	**1/6 (16,7%)**	20	25
Amoxicillin		0/4	16	24
Ampicillin	16 – 22	**1/7 (14,3%)**	16	24
Cefotaxime	29 – 35	**2/6 (33,3%)**	27	37
Cefoxitin		0/4	25	29
Ceftazidime	25 – 32	**2/6 (33,3%)**	24	33
Ceftiofur	26 – 31	**1/6 (16,7%)**	22	30
Chloramphenicol	21 – 27	0/7	22	27
Ciprofloxacin	30 – 40	0/7	30	40
Florphenicol	22 – 28	0/7	23	27
Gentamicin	19 – 26	0/7	20	26
Imipenem		0/3	27	31
Nalidixic acid	22 – 28	**1/7 (14,3%)**	21	28
Streptomycin	0 – 50	0/5	14	20
Sulfisoxazole	15 – 23	**3/6 (50%)**	6	26
Tetracycline	18 – 25	0/7	22	25
TMP+SMX		0/6	22	29
Trimethoprim	21 – 28	**1/5 (16,7%)**	20	27

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

- **219** test performed of which 7 were incorrect (deviation 3,2%)

Antimicrobial	QC range	Deviation/Total no of test (%)	Min value	Max value
Amoxicillin+clavulanic ac	2 – 8	0/3	4	8
Ampicillin	2 – 8	**1/18 (5,5%)**	1	8
Cefotaxime	0,03 – 0,12	**1/18 (5,5%)**	≤0,06	0,25
Cefoxitin		**1/2 (50%)**	4	26
Ceftazidime	0,06 – 0,5	0/13	≤0,25	0,25
Ceftiofur	0,25 – 1	0/5	≤0,25	0,5
Chloramphenicol	2 – 8	0/18	4	8
Ciprofloxacin	0,004 – 0,016	**3/17 (17,6%)**	≤0,08	0,03
Florphenicol	2 – 8	0/17	2	8
Gentamicin	0,25 – 1	0/18	≤0,25	0,5
Nalidixic acid	1 – 4	0/18	1	4
Streptomycin	4 – 16	**1/17 (5,9%)**	2	8
Sulfisoxazole	8 – 32	0/16	8	64
Tetracycline	0,5 – 2	0/18	1	2
TMP+SMX		0/3	<0,12	1
Trimethoprim	0,5 – 2	0/18	≤0,5	2

Summarizing *E. coli* trial

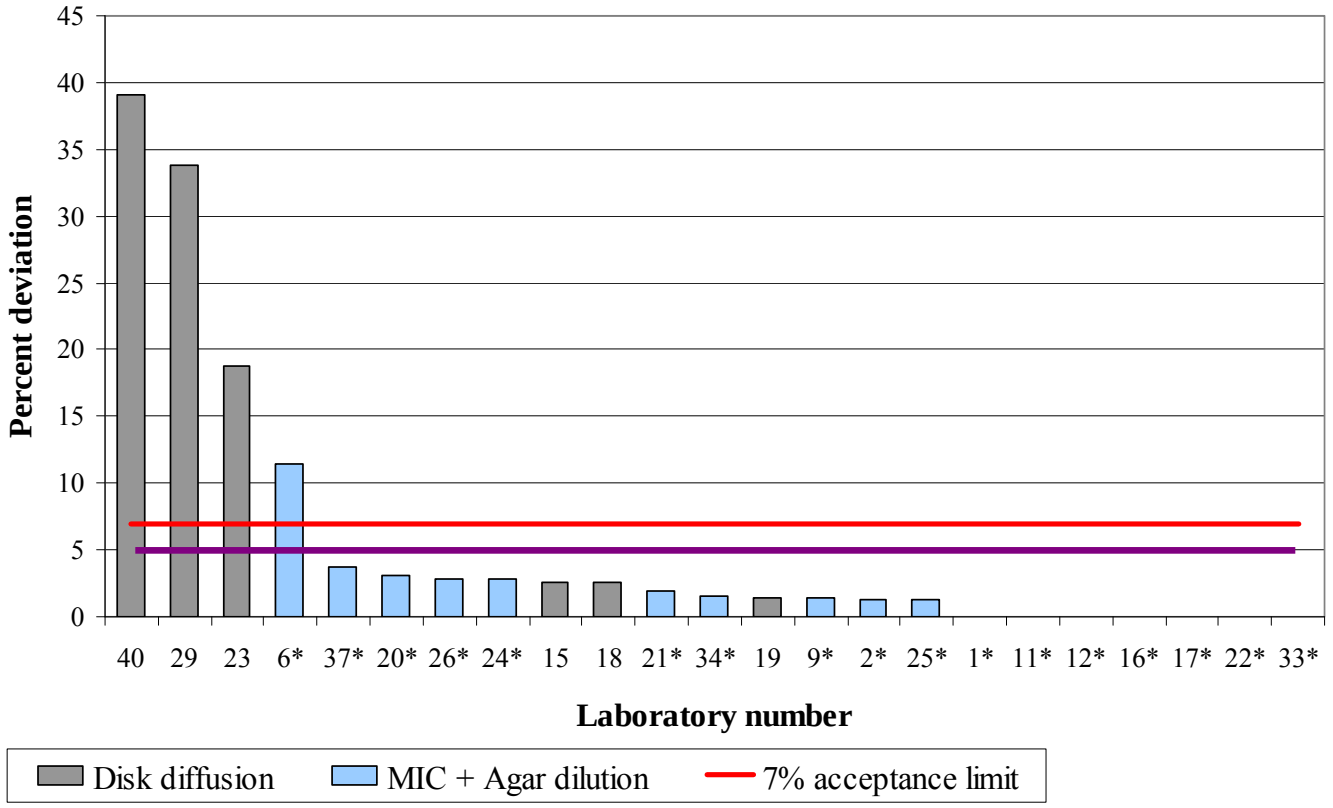
- One laboratory identified as outlier whereas the majority of the labs obtained deviations in the interval between 0%-1%
- Deviations were mainly caused by laboratories performing DD for AST
- 10/27 taking part in the *E. coli* trial obtained 100% of correct results, in 2007 only 6 participants achieved the 100%
- Discrepancy on the cut off values for ciprofloxacin
- The used of the double disk confirmatory test (CAZ/CL:CAZ and CTX/CL:CTX) appeared to be a successful test for identifying ESBL
- *For E. coli* ATCC 25922, the percentage of positive results for all test performed has increased from 90% in EQAS 2007 to 96.8% this year

Conclusions

- Performance has improved in the enterococci and staphylococci trial
    - enterococci needs attention regarding the antimicrobials recommended by EFSA
    - MRSA identification also needs attention (cause of major deviations for the staphylococci trial)
- *E. coli* trial deviation has suffered a small increase (0.1%)
    - 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 cut off values to define them as resistant
    - laboratories performing disk diffusion
- 4/29 participants have been categorised as outliers

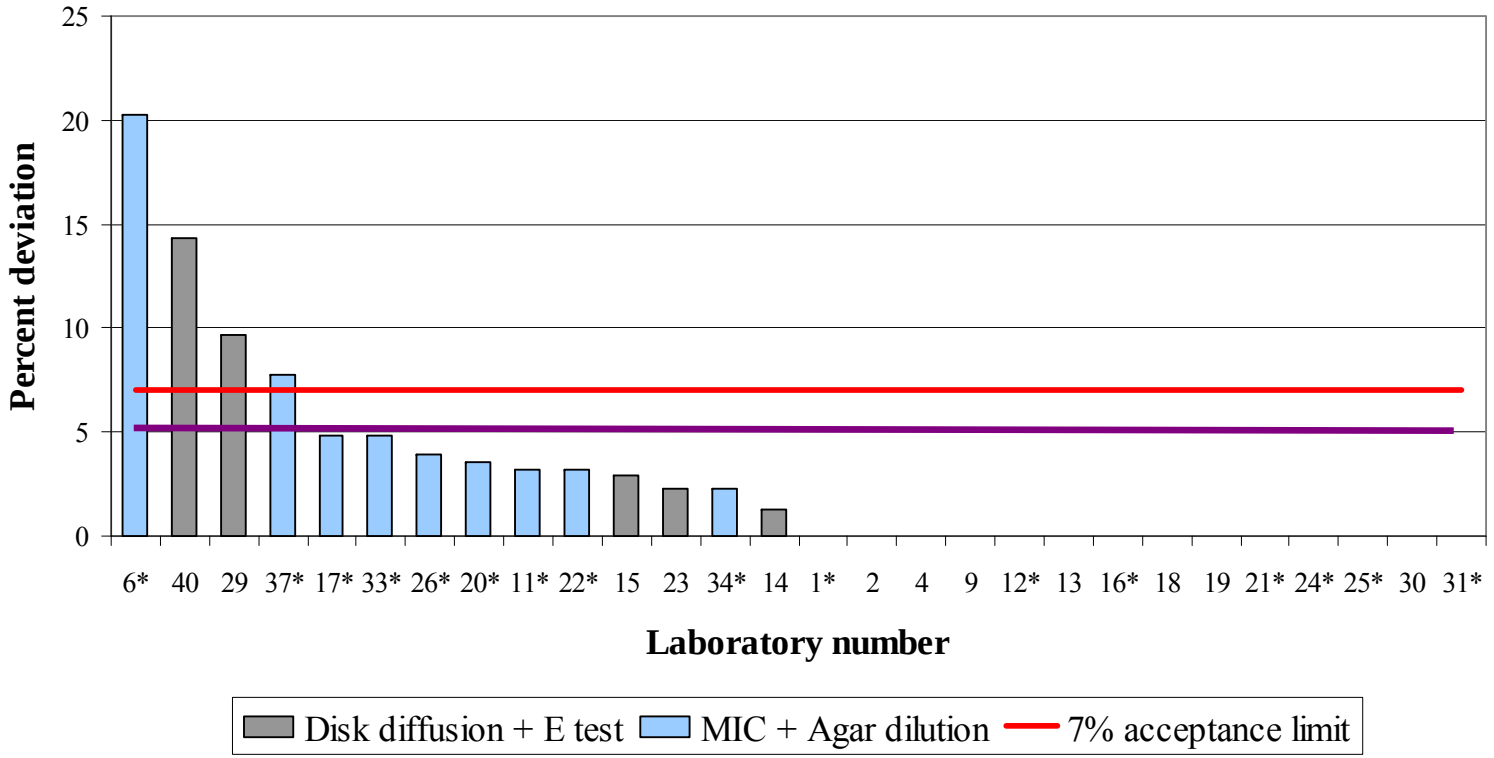
For next year: how about 5% deviation?

- For enterococci



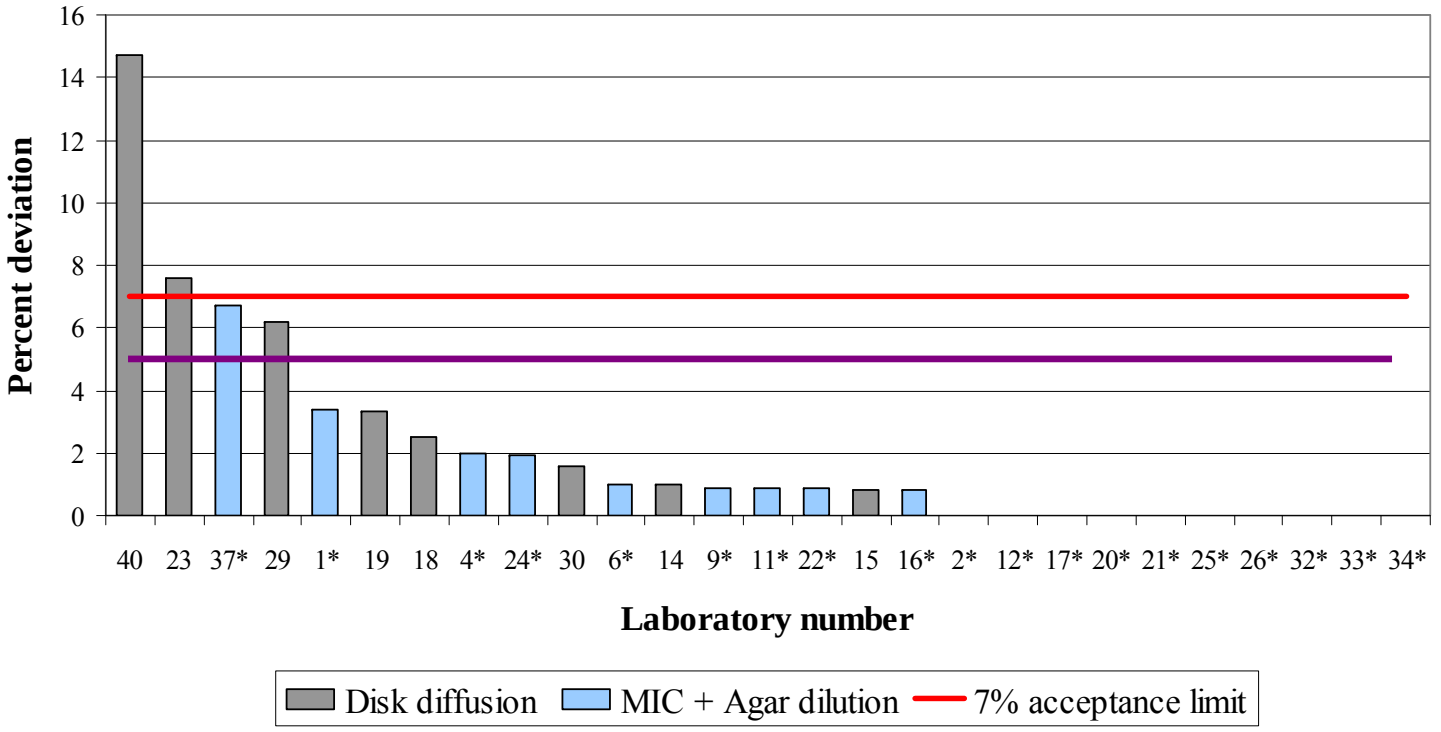
For next year: how about 5% deviation?

- For staphylococci



For next year: how about 5% deviation?

- For *E. coli*



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