



**EQAS 2007**

*E. coli*

**Enterococci**

**Staphylococci**



# CRL-AR EQAS

- The unit was in 2006 appointed Community Reference Laboratory (CRL) for Antimicrobial Resistance (AR) by the European Commission.
- One of the responsibilities as CRL is to conduct proficiency tests (EQAS) for the member states.

# EQAS

- The first EQAS for *Salmonella* + *Campylobacter* was carried out in 2006
- The second for Ent-Staph- *E.c* in June 2007
- The third for *Salmonella*+*Campylobacter* in October 2007
- The fourth for Ent-Staph- *E.c* has just been send out to the participants in June 2008

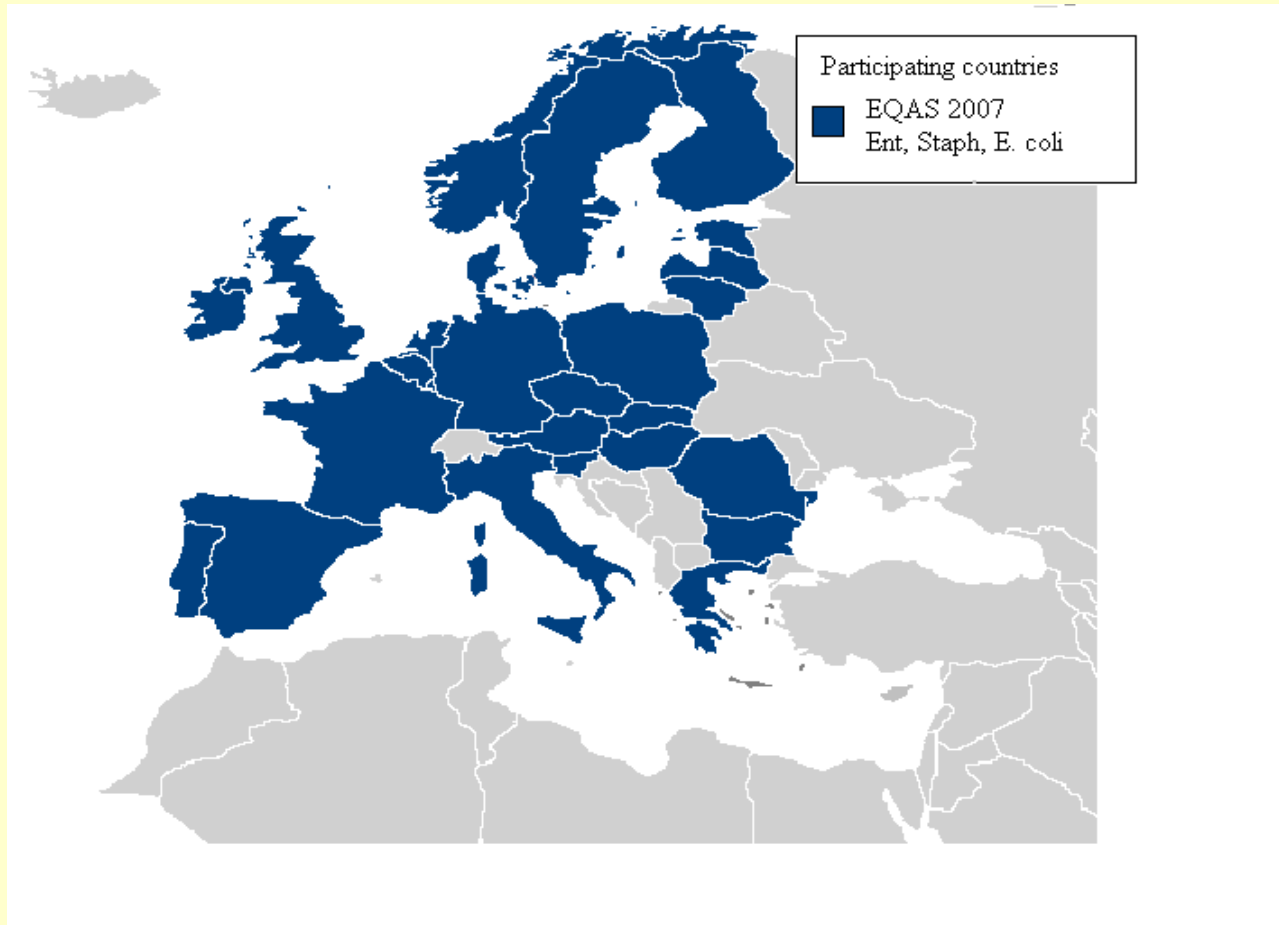
# Objective

- The objective of the EQAS is to monitor the quality of the antimicrobial susceptibility data produced by the member states
- Identify and support laboratories, which need guidance or assistance to produce reliable data

# Number of participants in the second EQAS 2007 (Ent-Staph- *E.c*)

- 25 member states
- 31 laboratories
- 26 laboratories analysed the Enterococci strains
- 31 the Staphylococci strains
- 30 the *E. coli* strains.

# Participating Countries



# Strains

- Eight strains of respectively Enterococci, Staphylococci and *E. coli* were selected for the EQAS from the Institute's strain collection.
- The antimicrobial susceptibility testing of the strains were performed by CRL-AR
- The obtained MIC values served as reference for the EQAS.
- U.S. FDA, Centre for Veterinary Medicine, verified the susceptibility patterns of the strains prior to distribution.

# Acceptance level

- CRL-AR has defined an acceptance level of a maximum of 7% deviations for each laboratory in the EQAS



# Reference strains

The laboratories were asked also to analyse

- *E. faecalis* ATCC 29212 (MIC)
- *S. aureus* ATCC 25923 (Disk)
- *S. aureus* ATCC 29213 (MIC)
- *E. coli* ATCC 25922 (MIC+Disk)

# Antimicrobials Enterococci

- Ampicillin α
  - Chloramphenicol α
  - Avilamycin
  - Ciprofloxacin
  - Daptomycin
  - Erythromycin α
  - Florfenicol
  - Gentamicin α
  - Linezolid α
  - Streptomycin α
  - Quinpristin-dalfopristin α
  - Tetracycline α
  - Tigecycline
  - Vancomycin α
- α **EFSA recommended antimicrobials to be included in the antimicrobial resistance monitoring.**

# Antimicrobials Staphylococci

- Chloramphenicol
- Ciprofloxacin
- Erythromycin
- Florfenicol
- Gentamicin
- Penicillin
- Streptomycin
- Sulfonamides
- Tetracycline
- Trimethoprim

EFSA does not recommend specific antimicrobials for resistance monitoring of Staphylococci

# Antimicrobials *E. coli*

Ampicillin $\alpha$	Ceftiofur	Tetracycline $\alpha$
Amoxicillin+ clavulanic acid	Chloramphenicol $\alpha$	Trimethoprim $\alpha$
Cefotaxime $\alpha$	Ciprofloxacin $\alpha$	Trimethoprim+ sulphonamides
Cefotaxime+ clavulanic acid	Florfenicol	
Cefoxitin	Gentamicin $\alpha$	$\alpha$ EFSA recommended antimicrobials to be included in the antimicrobial resistance monitoring.
Cefpodoxime	Imipenem	
Ceftazidime	Imipenem+ EDTA	
Ceftazidime+ clavulanic acid	Nalidixic acid $\alpha$	
	Streptomycin $\alpha$	
	Sulphonamides $\alpha$	

# Methods used

- MIC determinations at the Institute were performed using the Sensititre systems from Trek diagnostics Ltd.
- For the ESBL analysis of the *E. coli* strains was used E-test from AB-Biodisk.

# Cut off values

- The cut off values used by CRL-AR for the MIC results are developed by EUCAST and recommended by EFSA.
- EFSA does not recommend cut off values for disk diffusion.

## EFSA cut off values *E. coli*

MIC ( $\mu\text{g/mL}$ )

R is >

Amoxicillin cl., AUG	8
Ampicillin, AMP	8
Cefotaxime, CTX	0.25
Cefpodoxime, POP	1*
Ceftazidime, CAZ	0.5
Ceftiofur, XNL	1
Chloramphenicol, CHL	16
Ciprofloxacin, CIP	0.032
Florfenicol, FFN	16
Gentamicin, GEN	2
Nalidixic acid, NAL	16
Streptomycin, STR	16
Sulfonamides, SMX	256**
Tetracycline, TET	8
Trimethoprim, TMP	2
Trimethoprim + sulfamethoxazole, TMP+SMX, SXT	0,5 15

\* Tentative \*\* CLSI

## Cut off values Enterococci

	MIC ( $\mu\text{g/mL}$ ) R is > <i>E. faecium</i>	MIC ( $\mu\text{g/mL}$ ) R is > <i>E. faecalis</i>
Ampicillin, AMP	4	4
Avilamycin, AVI	16	8
Chloramphenicol, CHL	32	32
Ciprofloxacin, CIP	4	4
Daptomycin, DAP	4	4
Erythromycin, ERY	4	4
Florfenicol, FFN	8	8
Gentamicin, GEN	32	32
Linezolid, LZD	4	4
Streptomycin STR	2048	2048
Quinpristin-dalfopristin, SYN	4	32
Tetracycline, TET	2	2
Tigecycline, TGC	0,25	0,25
Vancomycin, VAN	4	4



## Cut off values for *S. aureus*

	MIC ( $\mu\text{g/mL}$ ) R is >
Chloramphenicol, CHL	16
Ciprofloxacin, CIP	1
Erythromycin, ERY	1
Florfenicol, FFN	8
Gentamicin, GEN	1
Penicillin, PEN	0,25
Streptomycin, STR	16
Sulfonamides, SMX	128
Tetracycline, TET	1
Trimethoprim, TMP	4

# Disk diffusion break points

- Participants using disk diffusion in this EQAS were recommended to interpret the results according to the individually routinely used breakpoints and categorise the results only as resistant or sensitive.
- The laboratories were also asked to submit the breakpoints used to the web-based database

## Methods used by EQAS-participants

- In the Enterococci trials:
- 15 laboratories used MIC determination
- 11 laboratories used disk diffusion.

## Methods used by EQAS-participants

- In the Staphylococci trials,
- 14 laboratories used MIC determination,
- 2 used E-test
- 15 laboratories used disk diffusion.

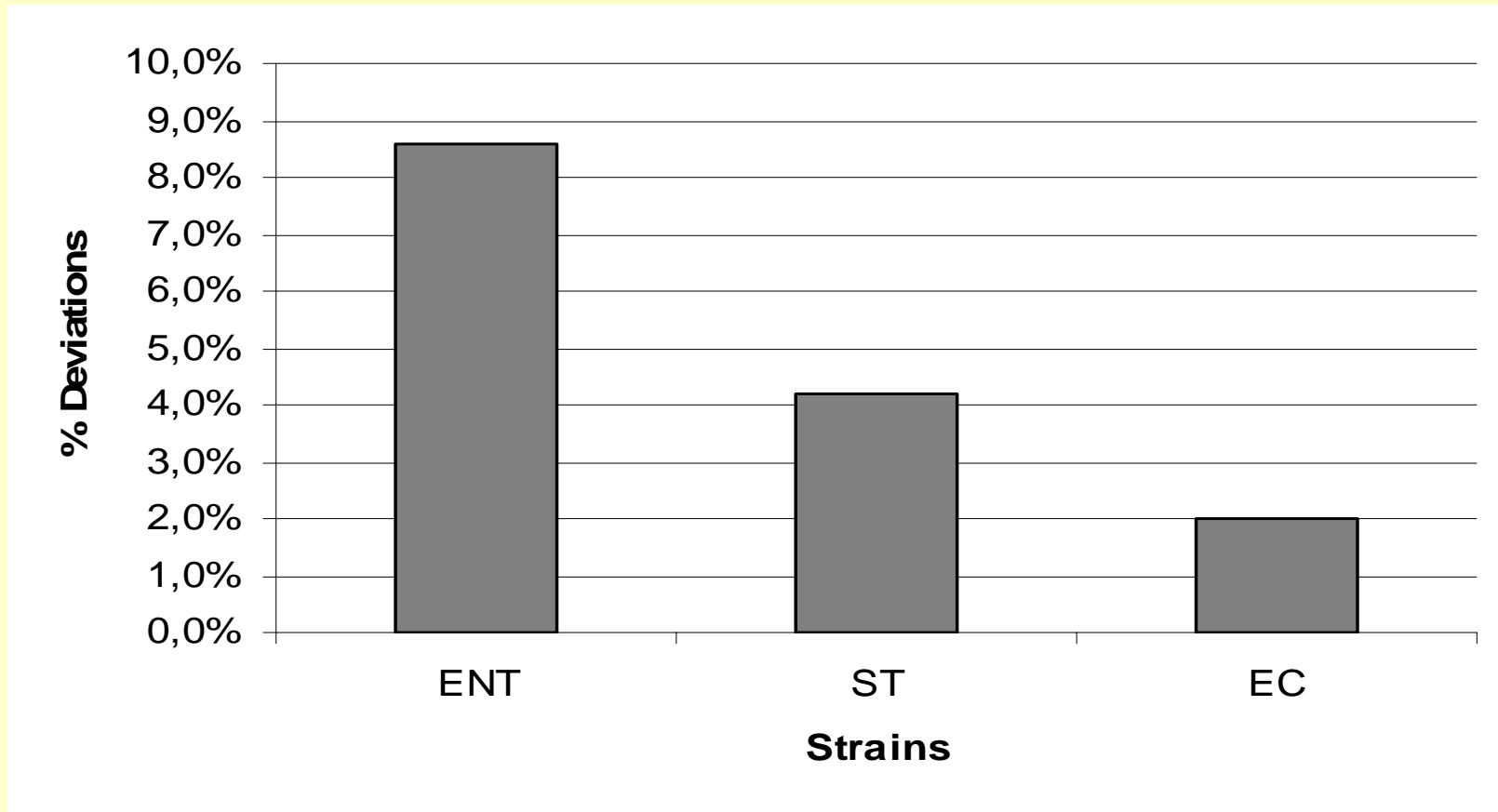
## Methods used by EQAS-participants

- In the *E. coli* trials:
- 15 laboratories used MIC determination
- 1 used E-test
- 14 laboratories used disk diffusion

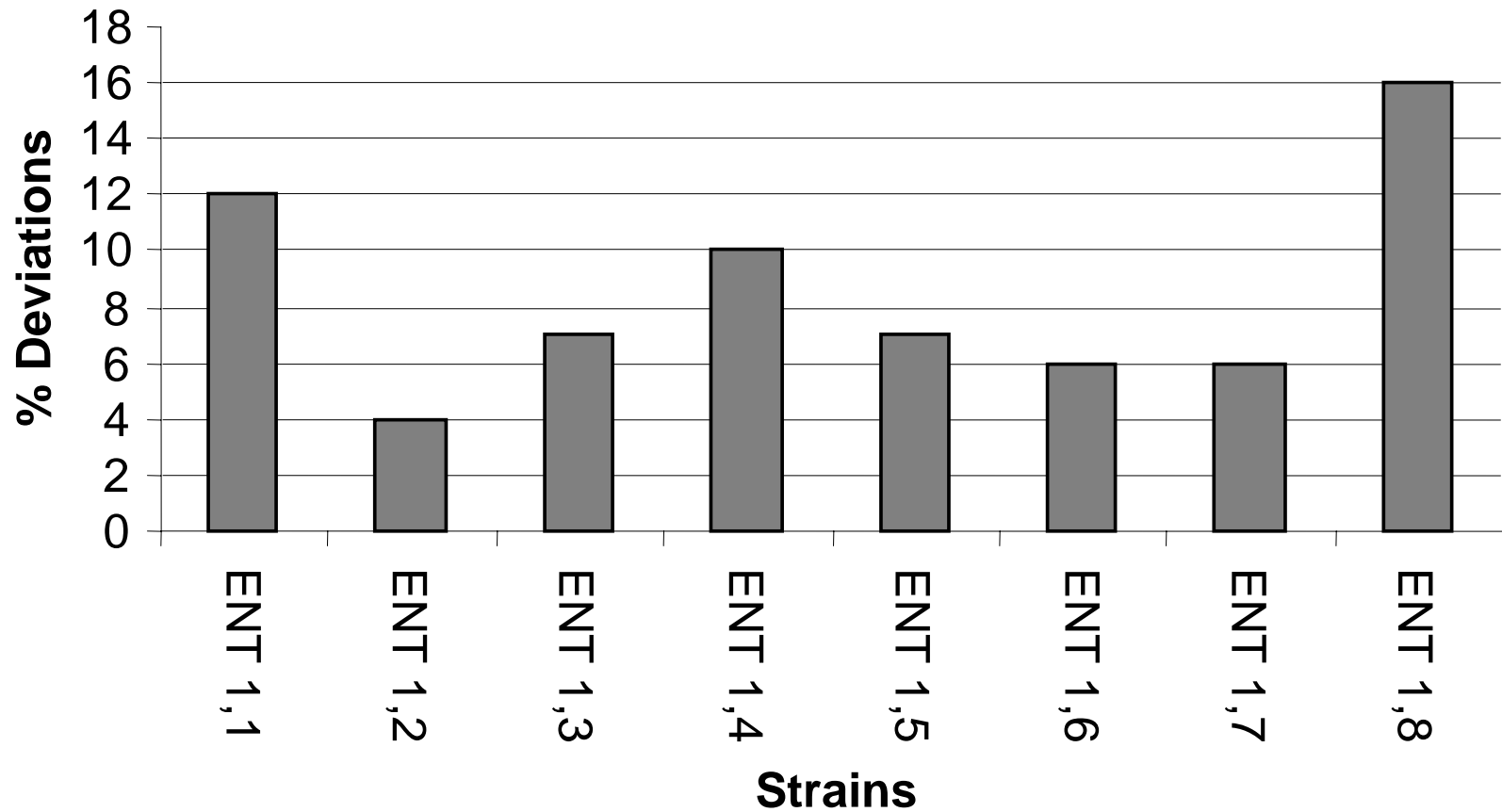
## **% Correct results by strains**

- Enterococci (ENT) strains 91.4% correct results
- Staphylococci (ST) strains 95.8% correct results
- *E. coli* (EC) strains, 98.0% correct results

# %Deviations per strain

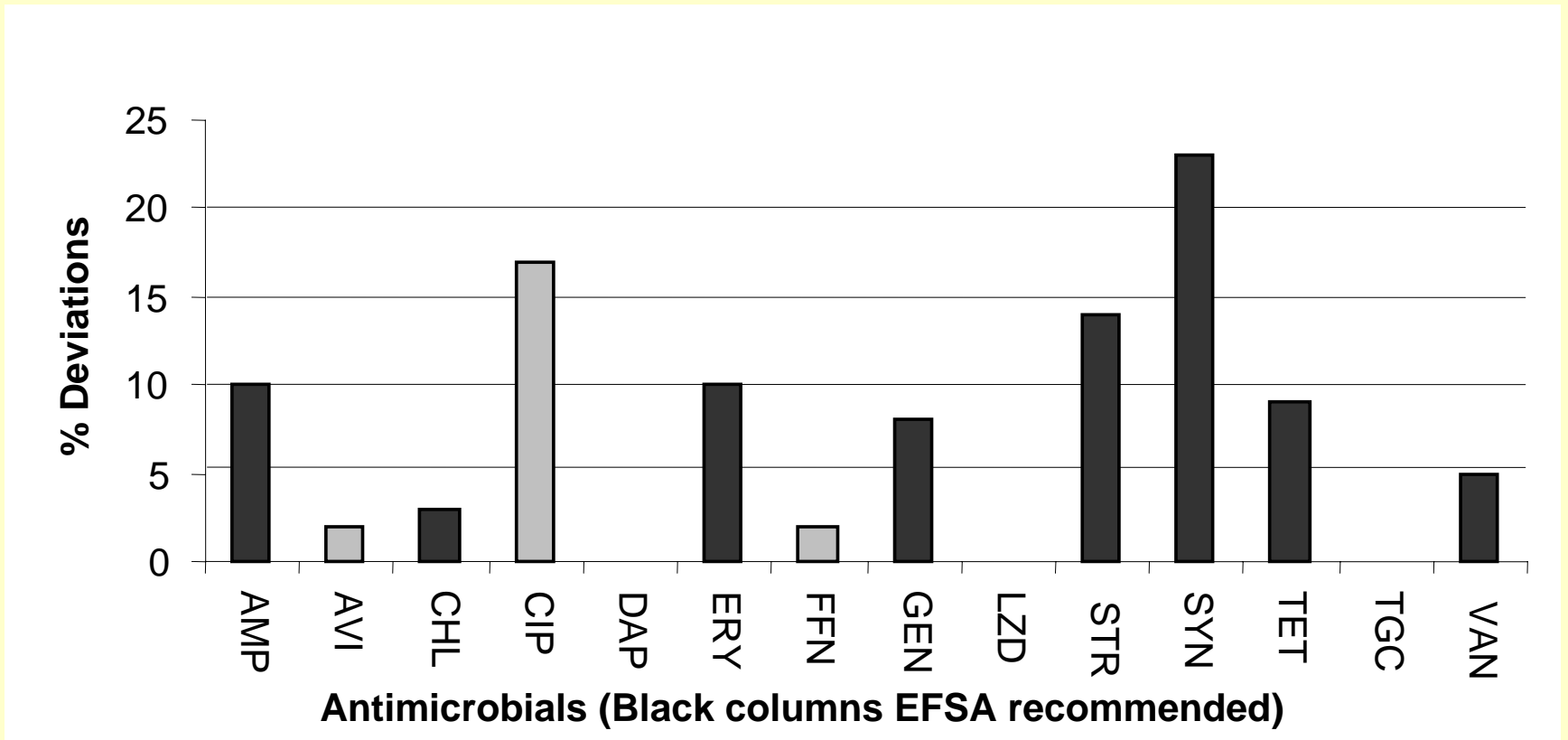


## Deviations Enterococcus strains





# Deviations antimicrobials Enterococci

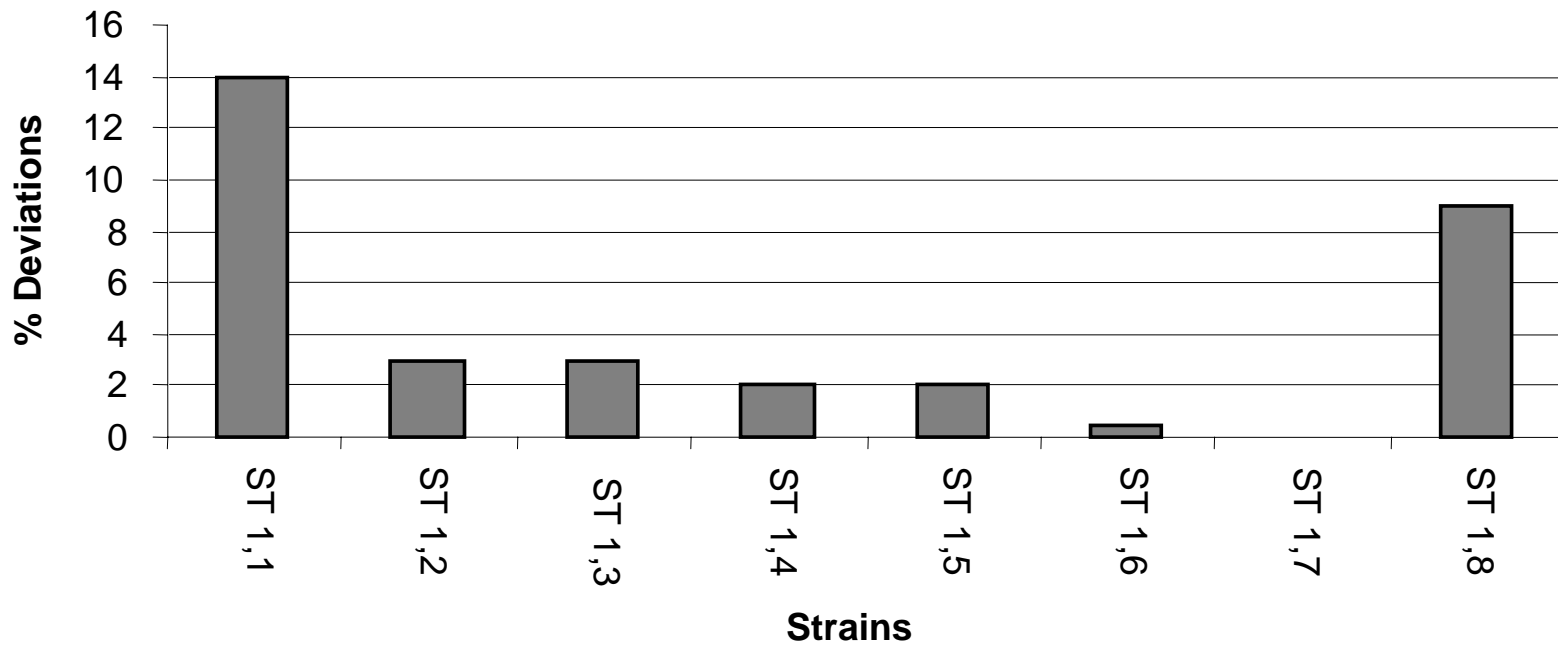


# Cause of deviation antimicrobials

## Enterococci

- The deviations for ciprofloxacin (not included in the EFSA recommended panel) and quinpristin-dalfopristin were very high
- Ciprofloxacin can in some cases be considered as borderline by disk diffusion
- Quinpristin-dalfopristin has two different break points, for *E. faecium* and *E. faecalis*, respectively. This might have caused misinterpretations

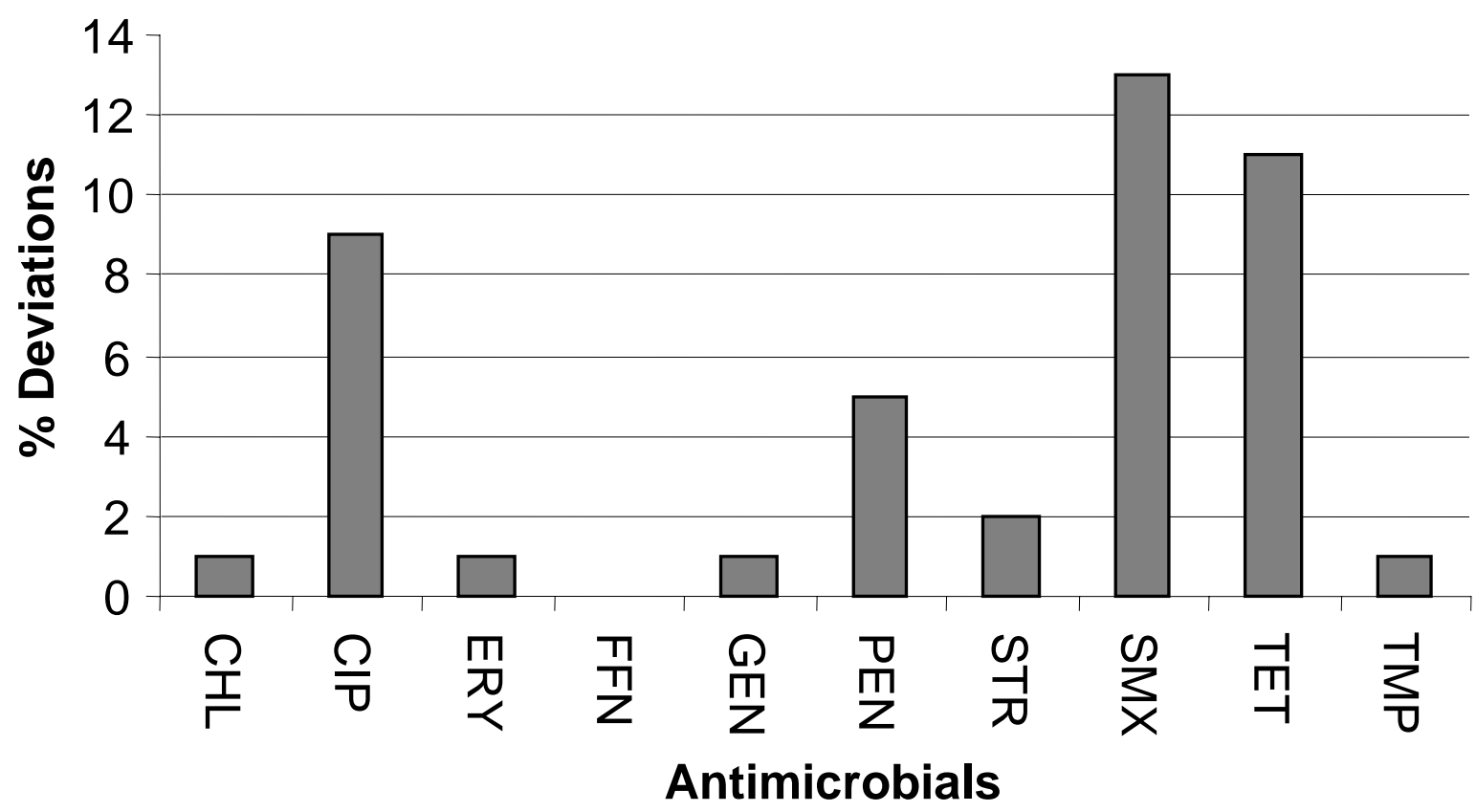
# Deviations Staphylococcus strains



# Cause of deviation *Staphylococcus* strain

- Strain ST 1.1. is resistant to methicillin and therefore according to CLSI should be interpreted resistant to penicillin
- The MIC value for ST1.1. for penicillin is 0.12
- The cut off value for penicillin is 0.25

# Deviations antimicrobials Staphylococci



# Cause of deviations in antimicrobials

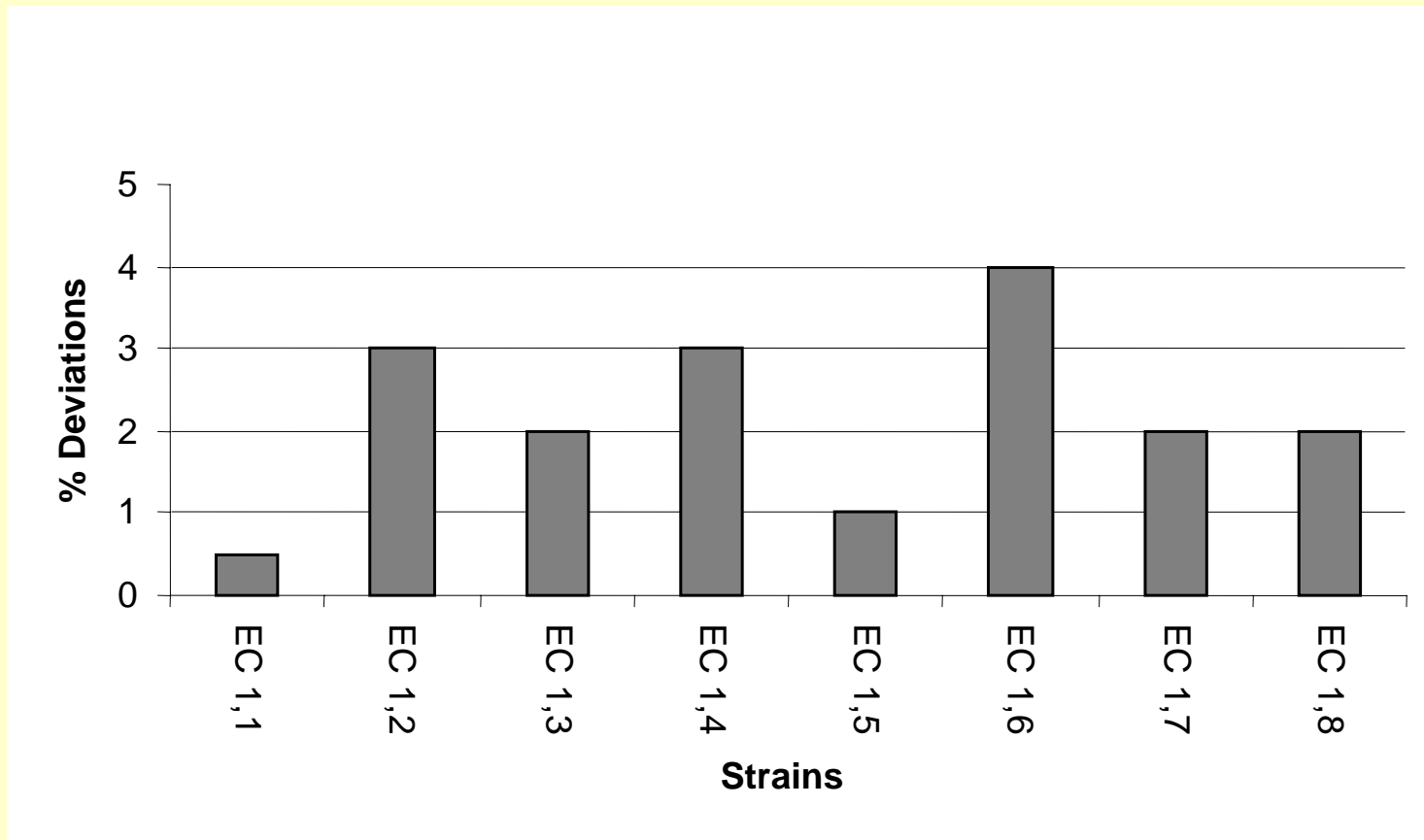
## Staphylococci

- The high percentage of deviations in sulphonamide could be observed in five of the eight strains
- The interpretation of results from disk diffusion of sulphonamide is difficult because of false negative resistance and double zone on the agar.

# MRSA

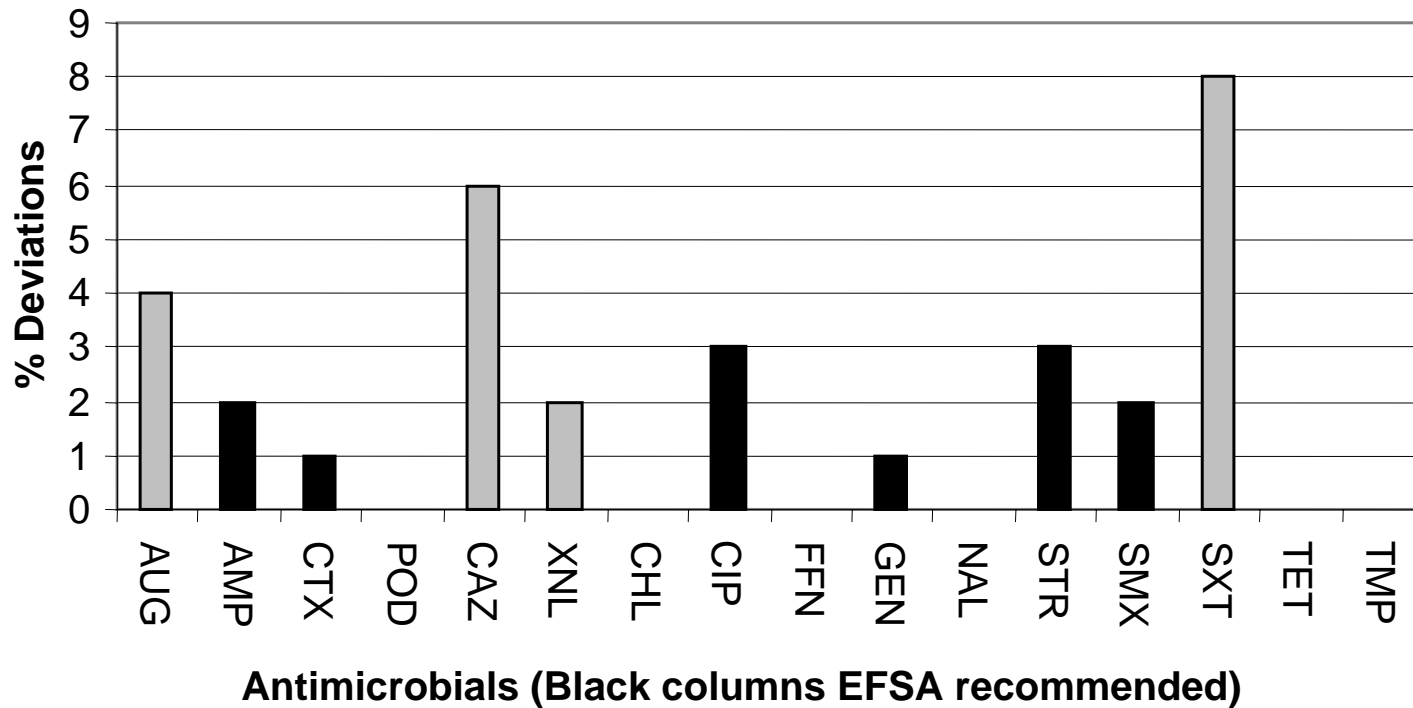
- The participants could optionally analyse for MRSA positive strains
- Strain ST 1.1 and ST 1.8 were MRSA positive
- Twenty three laboratories analysed the strains for MRSA
- Four (17%) laboratories did not identify the strains correctly as MRSA

# Deviations *E. coli* strains





# Deviations antimicrobials *E. coli*



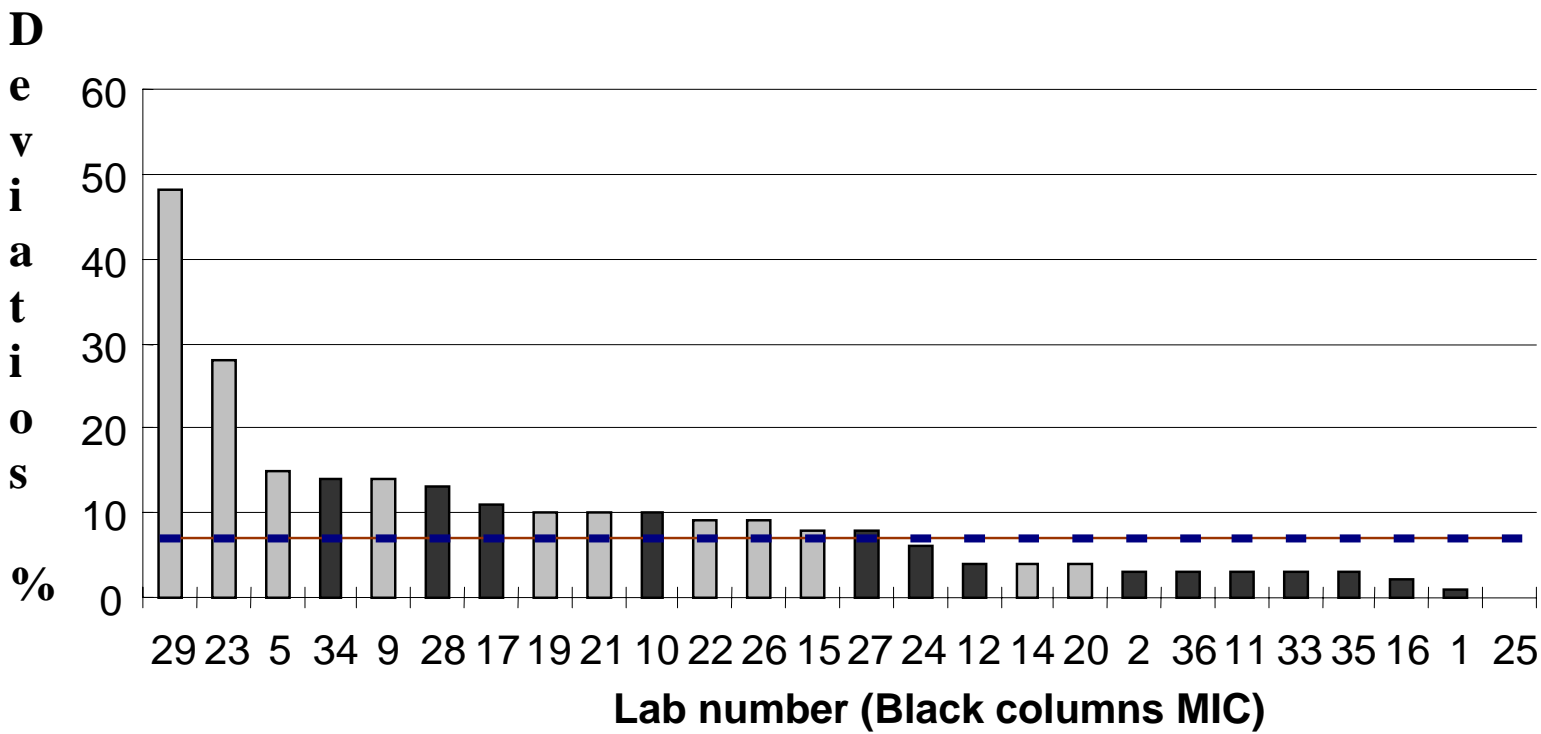
# ESBL

- The laboratories could optionally analyse for the ESBL producing *E. coli* strains.
- The protocol specify that a strain should be interpreted resistant to all cephalosporin if it is interpreted resistant to one.
- Strain EC 1.6 was ESBL producing.
- Eighteen laboratories analysed the *E. coli* strains for ESBL production and all identified strain EC 1.6 correctly.
- One laboratory incorrectly identified strain EC 1.7 as ESBL producing

# AmpC

- Strain EC 1.7 was AmpC positive.
- Eight out of nine laboratories, which analysed the EC-strains for AmpC, detected correctly the strain as AmpC.
- One lab analysed the strain as non-AmpC.
- None of the nine laboratories, which performed the AmpC analyse, analysed the strain as ESBL.

# % Deviations for Enterococci per lab



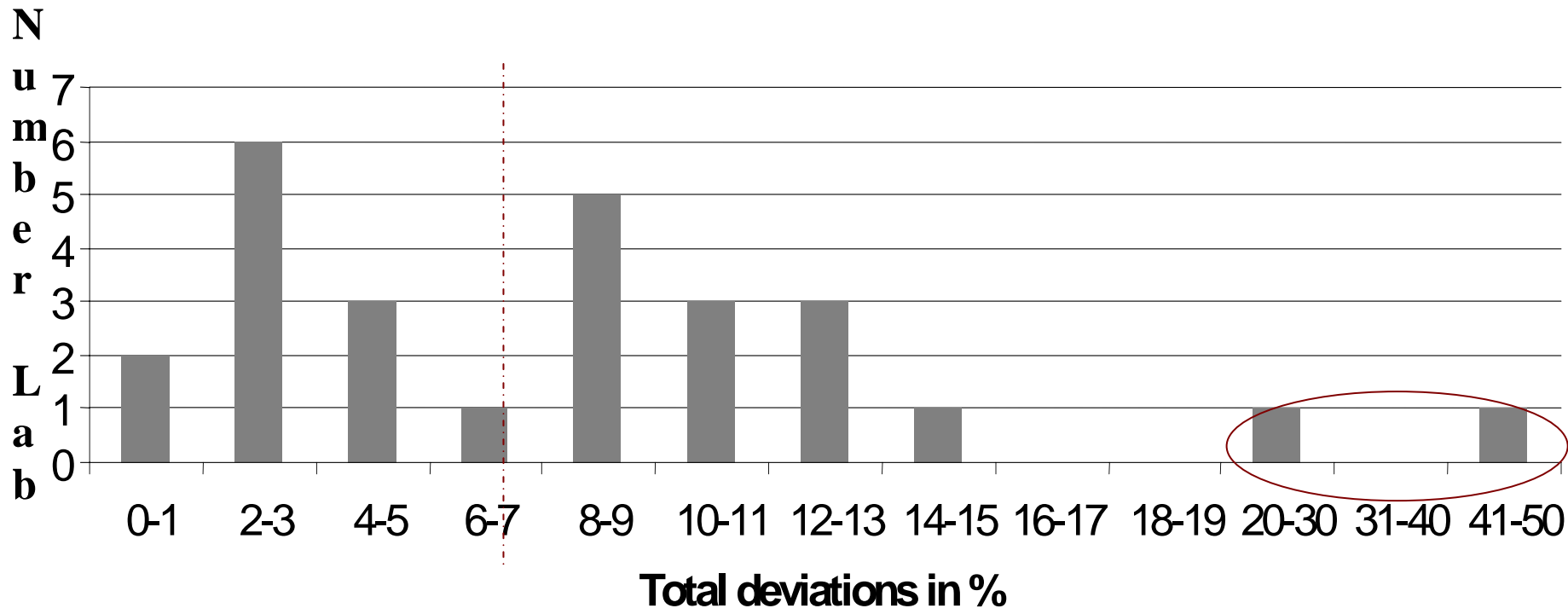
## Summary Enterococci

- Fourteen laboratories had  $> 7\%$  deviations in the AST (CRL-AR defined acceptance level).
- The percentage of deviations differed widely between the laboratories with a maximum of 48% deviations to a minimum of 0% deviations.

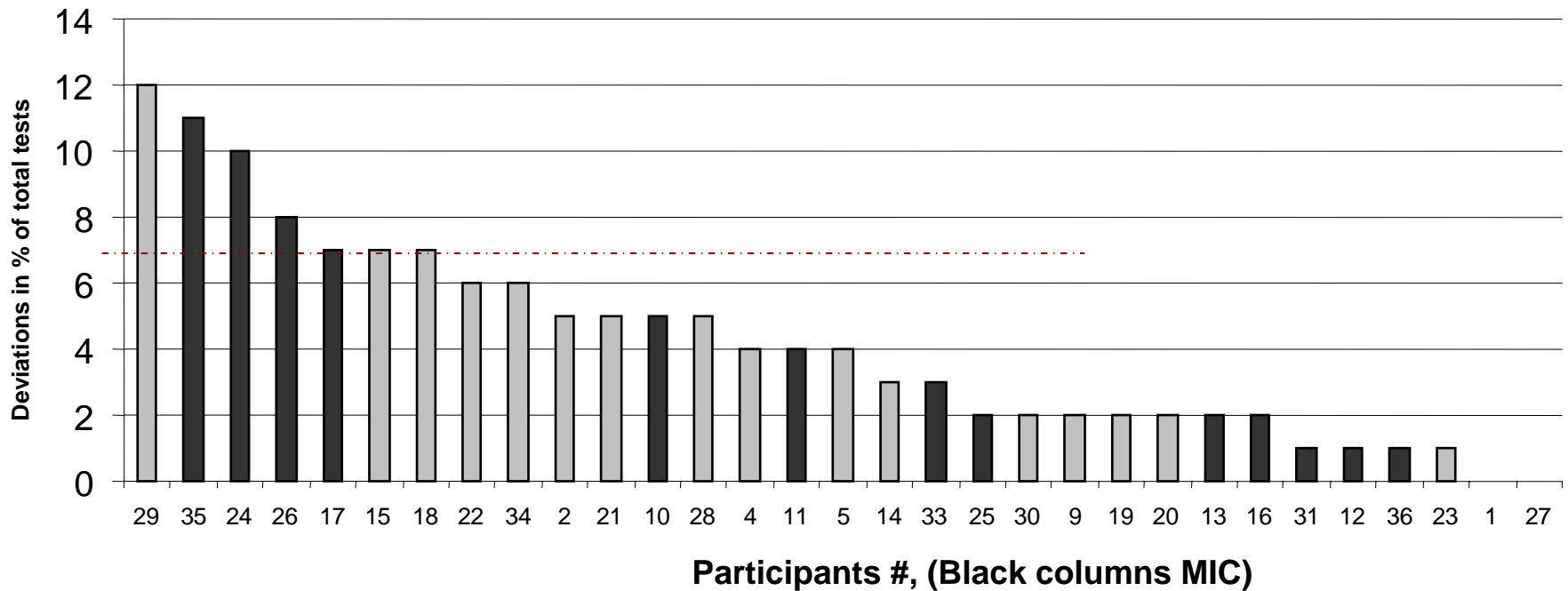
## Disk/MIC

- Nine of eleven laboratories with  $> 7\%$  deviations used disk diffusion
- Four of fourteen laboratories with  $> 7\%$  deviations used MIC determination
- The results obtained by using MIC was significantly better than the results using disk

# Number of labs listed in intervals of % per total deviation for Enterococci



# %Deviations for Staphylococci per lab





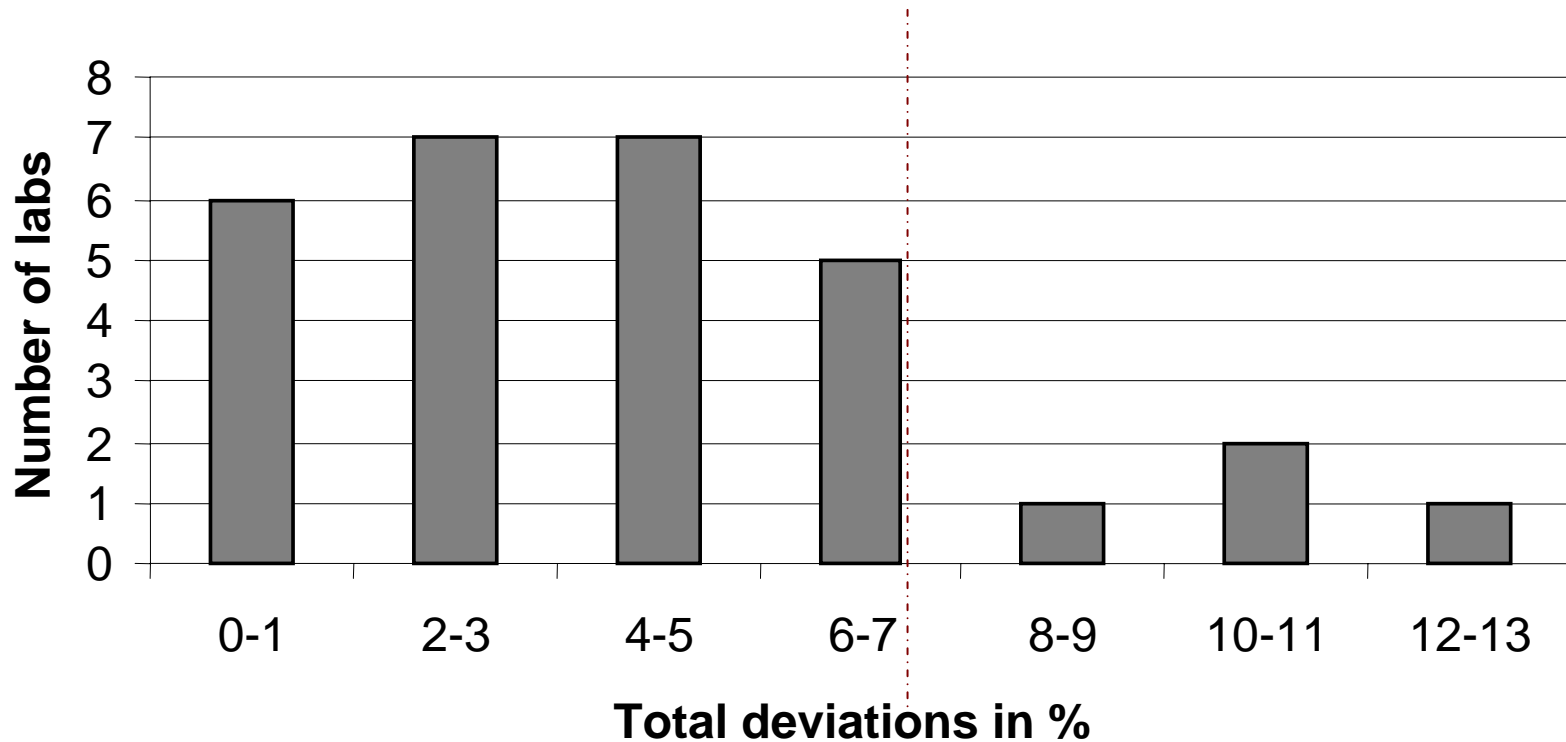
## Summary Staphylococci

- Four laboratories had  $> 7\%$  deviations in the AST
- The percentage of deviations differed between the laboratories from 12 % to 0%

## Disk/MIC

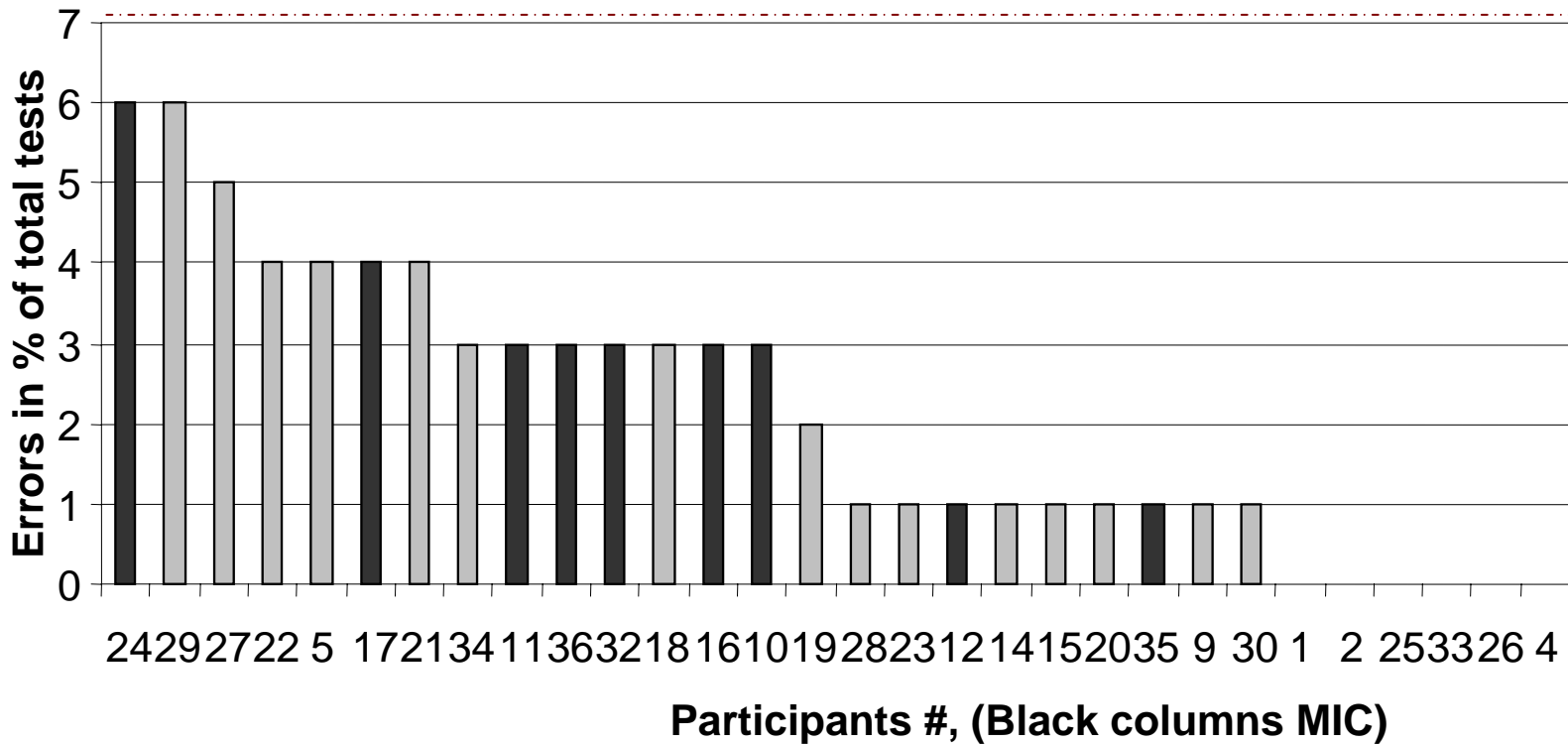
- One of fifteen laboratories with  $> 7\%$  deviations used disk diffusion
- Three of fourteen laboratories with  $> 7\%$  deviations used MIC determination.
- There is no significant difference between the methods.

## Number of labs listed in intervals of % per total deviation for Staphylococci





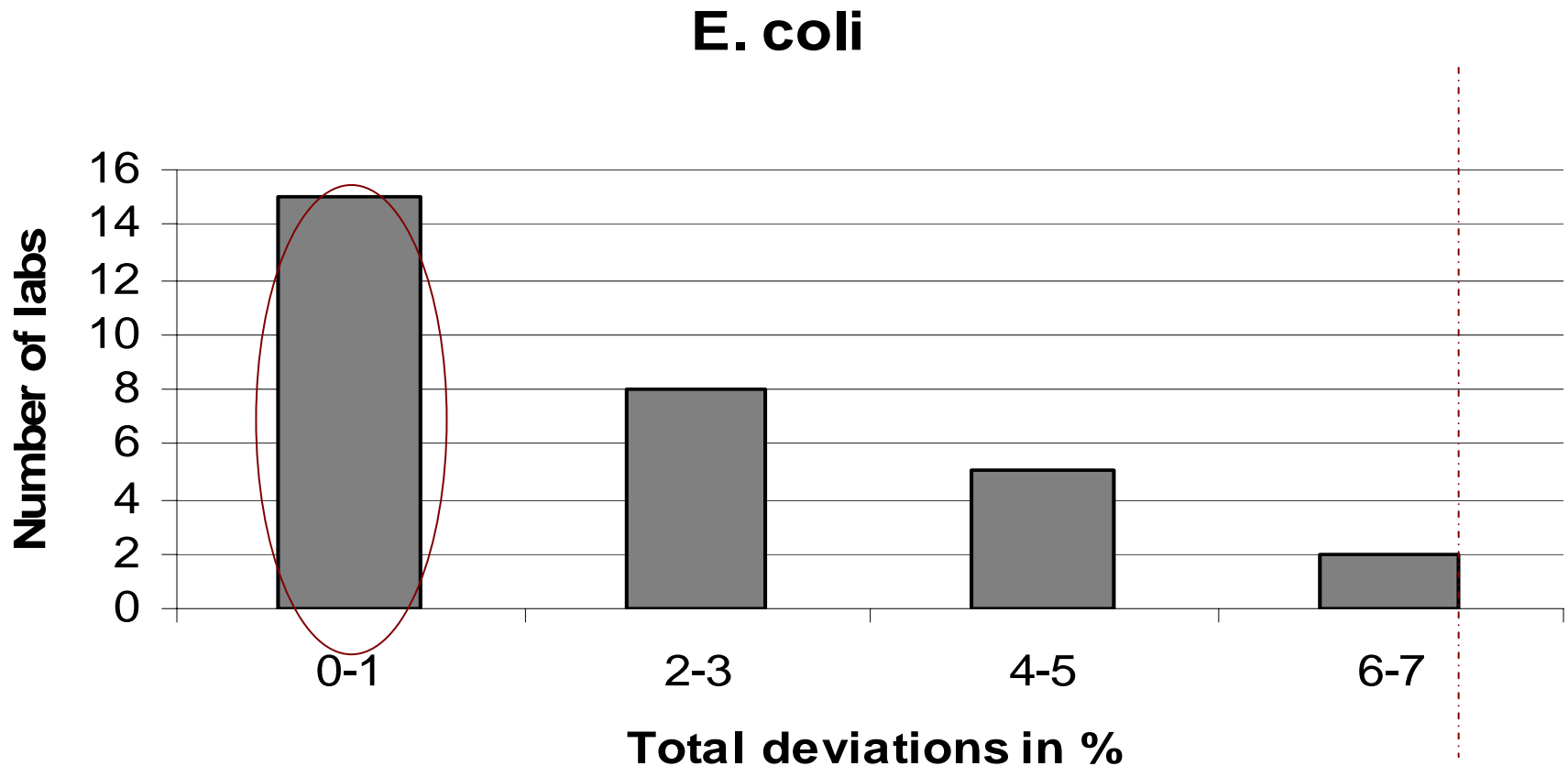
# % Deviations per lab for E. coli



## Summary *E. coli*

- Six of the laboratories obtained a result of 100 % correctly tested *E. coli* strains.
- All 30 labs showed <7 % deviations.
- There is no significant difference between the results obtained by disc diffusion and MIC determination.

## Number of labs listed in intervals of % per total deviation for *E.coli*





# Reference strains

- The analyses of the reference strains showed surprisingly divergent results compared to the AST.



# Reference strain Enterococci

- The AST of the Enterococci strains obtained the greatest deviation on 9% overall
- The test of the reference strain obtained 2% deviations in MIC testing.





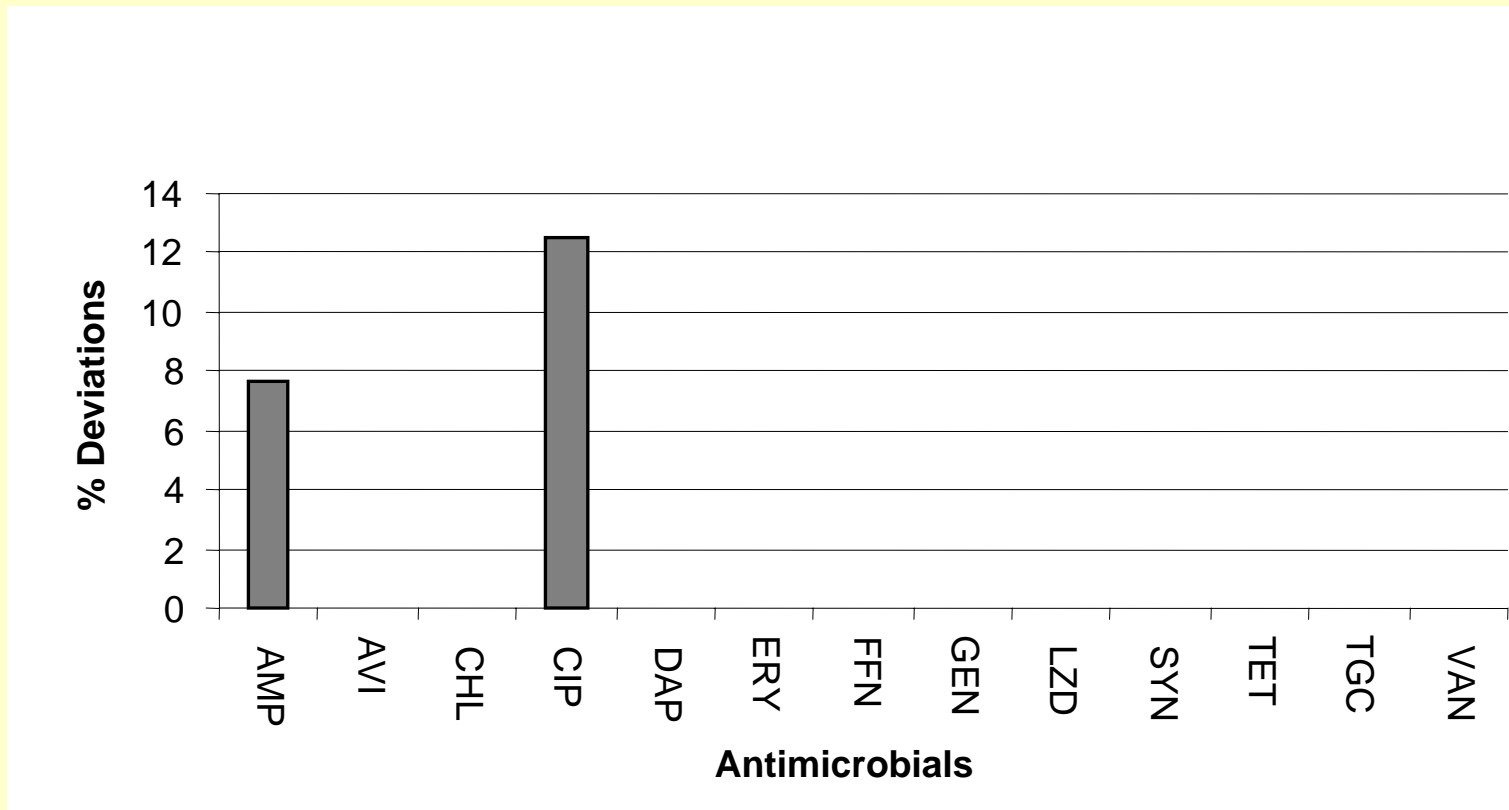
## Reference strain Staphylococci

- The AST of the Staphylococci strains obtained 4% deviations overall
- The test of the reference strain obtained:
  - 18% deviations in disk diffusion
  - 6% in MIC testing.

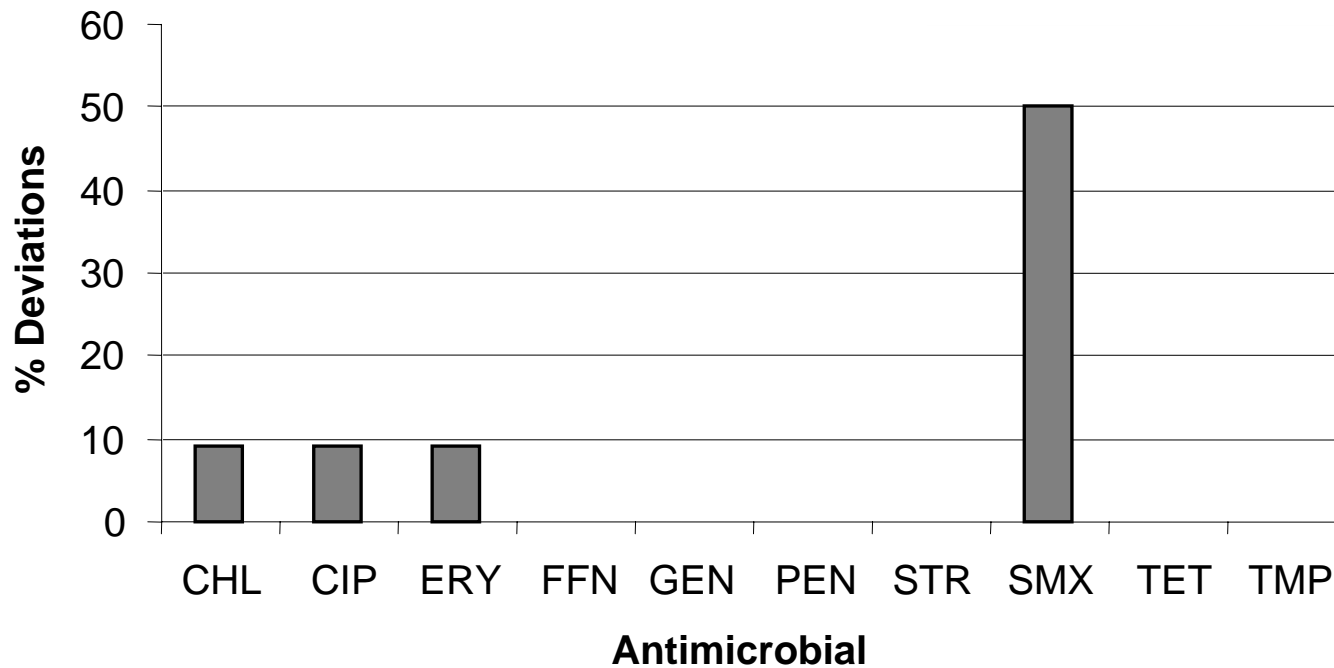
## Reference strain *E. coli*

- The AST of the *E. coli* strains obtained 2% deviations overall while
- The test of the reference strain obtained 10% deviations in both disk diffusion and MIC.

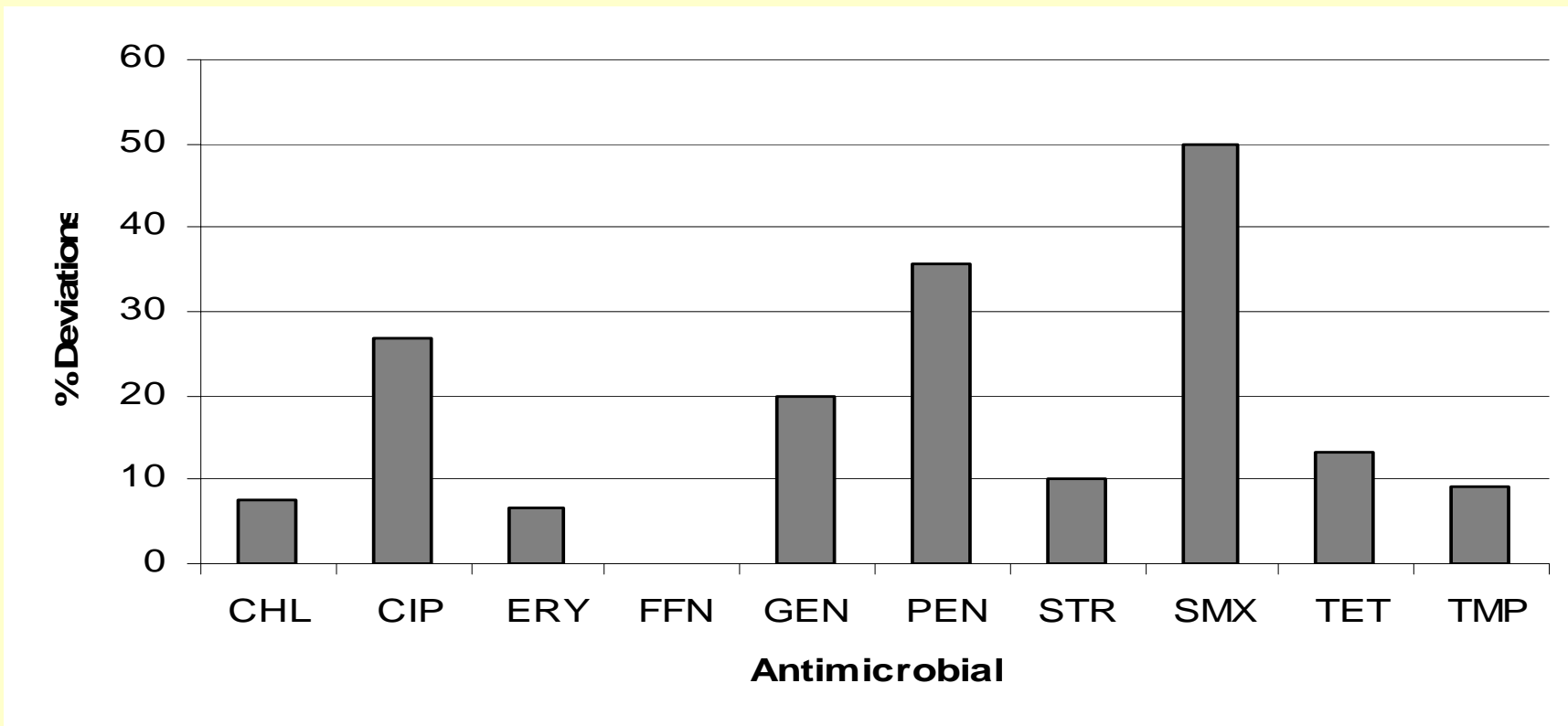
# Deviations *E. faecalis* ATTC 29212 MIC



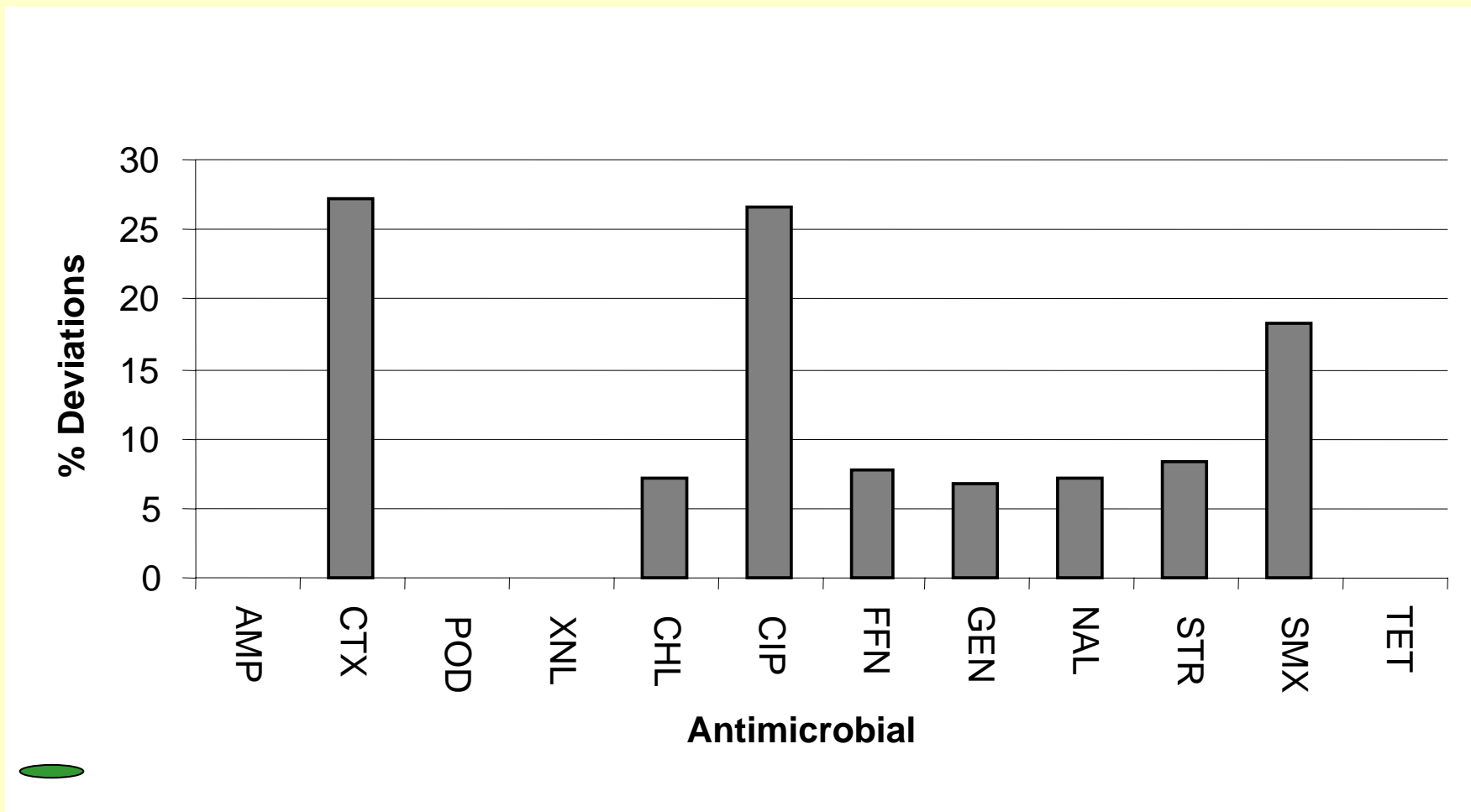
# Deviations ATTC *S. aureus* 25913 MIC



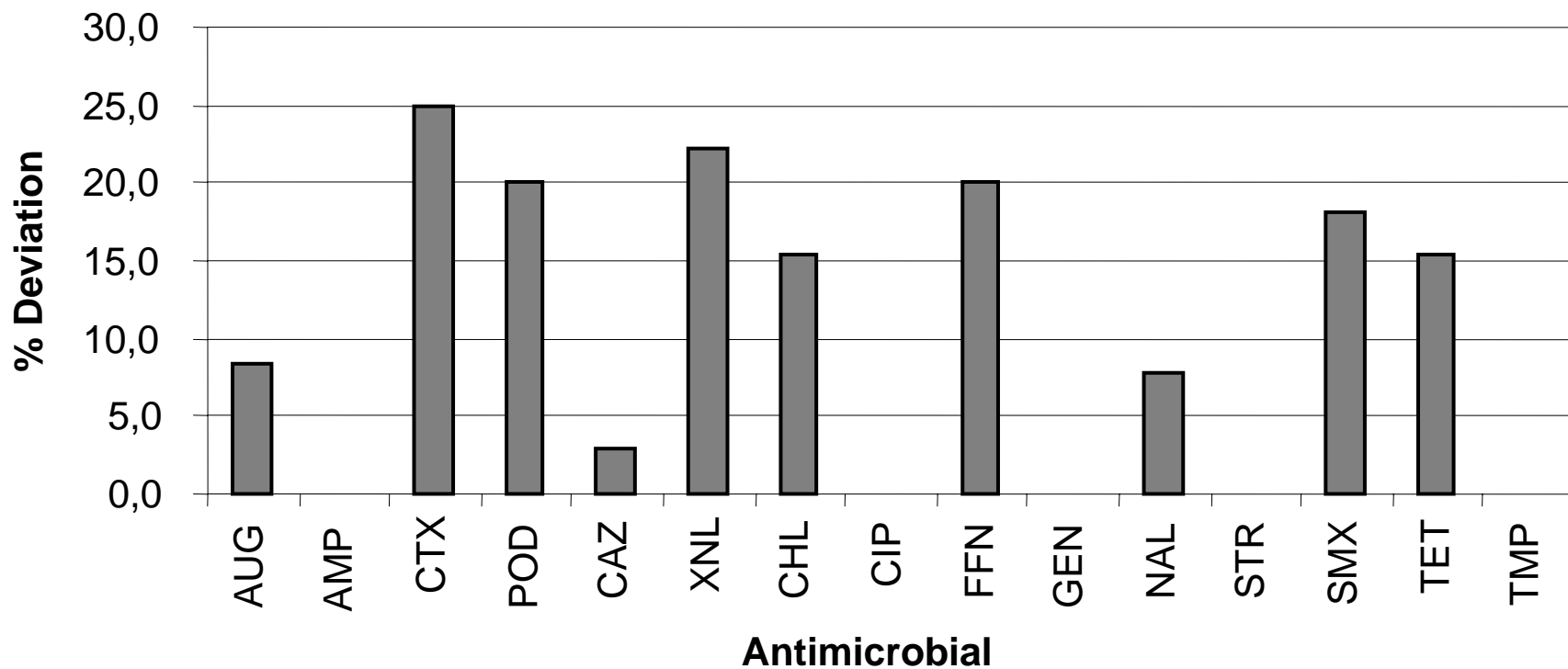
# Deviations *S. aureus* ATTC 25923 disk diffusion



## Deviations *E. coli* ATTC 25922 MIC



# Deviations E. coli ATTC 25922 disk diffusion



# Reference strain

- The reason for the larger deviations in reference strain testing than in AST of the strains is that the margin for success is much smaller in testing reference strains. It is the same fact that makes reference testing a very useful tool for determining errors in the procedures and quality assurance.
- It is therefore of paramount importance that all participants submit results from testing the reference strains.



# Conclusion

- The goal of the CRL-AR is that all laboratories perform susceptibility testing with a deviation margin below 7% in the EQAS and as a consequence that all NRLs generate correct and reliable data on a routine basis.
- The performance of AST of Enterococci needs considerable improvement to reach the goal, while the goal for Staphylococci is closer at hand and is already accomplished for *E. coli*

## Conclusion

- It is encouraging that the laboratories performed so well in detecting the ESBL producing *E. coli*-strain and obtained 94% correct results,
- There still is room for improvement regarding the MRSA analysis where 17% of the laboratories obtained deviating results.

## Future goals

- The most important issues to address in the future collaboration between the CRL-AR and the NRL's is the harmonisation of breakpoints as well as the choice of antimicrobials and methods.
- The goal is that the participants in analysing the strains carry out the criteria set by EUCAST/EFSA for AST as agreed on at the CRL-AR Workshop in Copenhagen in May 2007