



DTU Food
National Food Institute

EQAS 2008

Salmonella and Campylobacter

CRL workshop, April 23-24, 2009

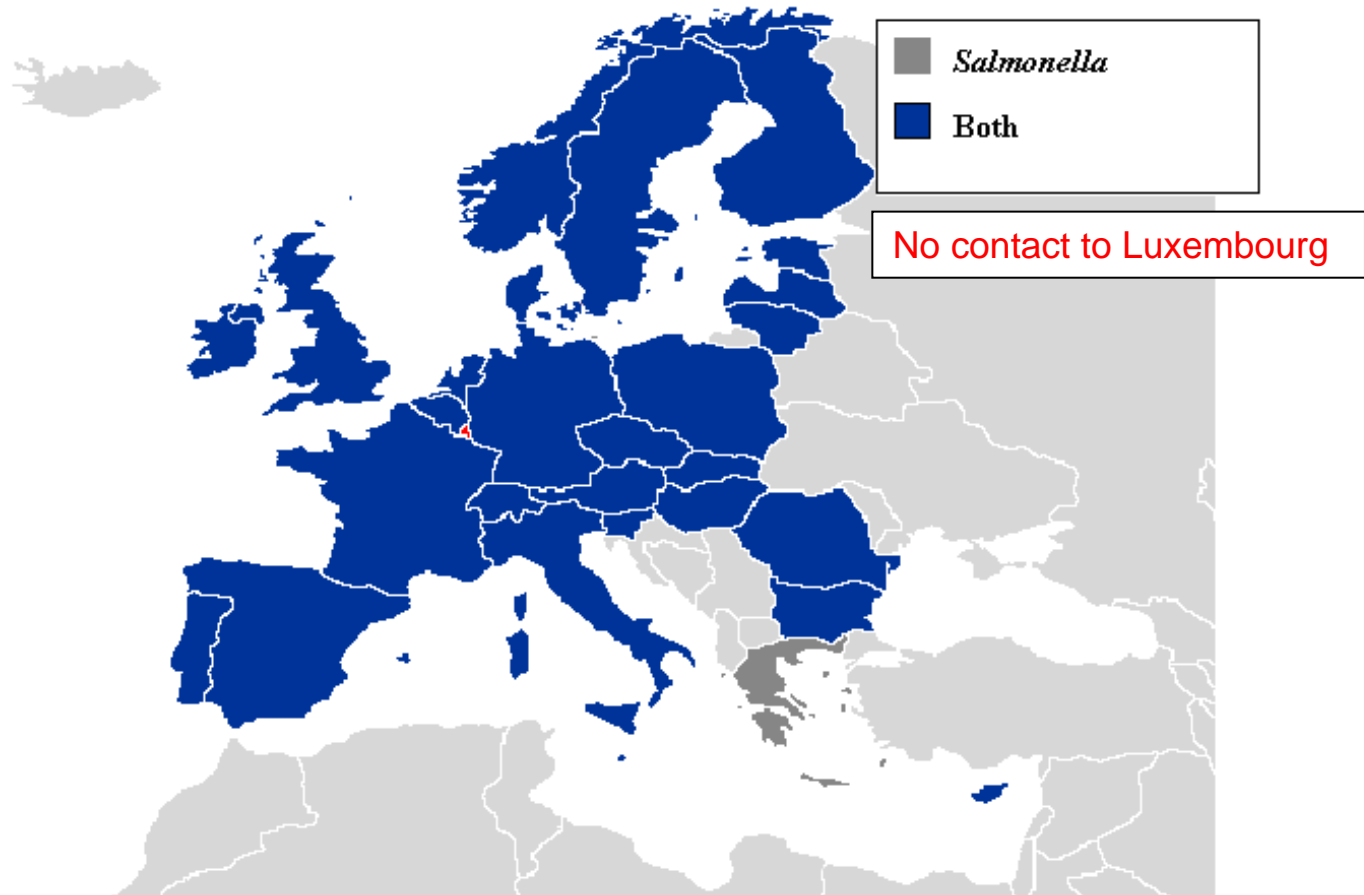
Susanne Karlsmose (suska@food.dtu.dk)

Outline of Salm/Camp EQAS 2008

- AST of eight *Salmonella* and eight *Campylobacter*
- New participants were supplied with original reference strains ATCC 25922 and ATCC 33560 for QC testing
- Participants' results and comments submitted through a secured web-based data entry program, using individual logins and passwords
- Instant individual evaluation report
- Report comparing and evaluating all results
- If only 75% of the results were correct (strain/antimicrobial combination), results should be further analysed, possibly omitted



Participation in the Salm/Camp EQAS, 2008



Issues important for differences in performance

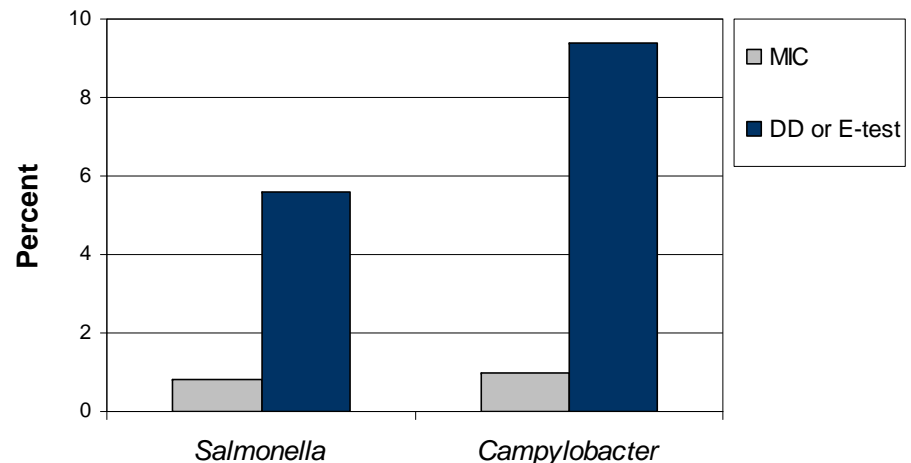
Goal for this EQAS: that all NRL's perform AST with less than 7% incorrect interpretations

Further investigation of a laboratory's deviations includes looking into:

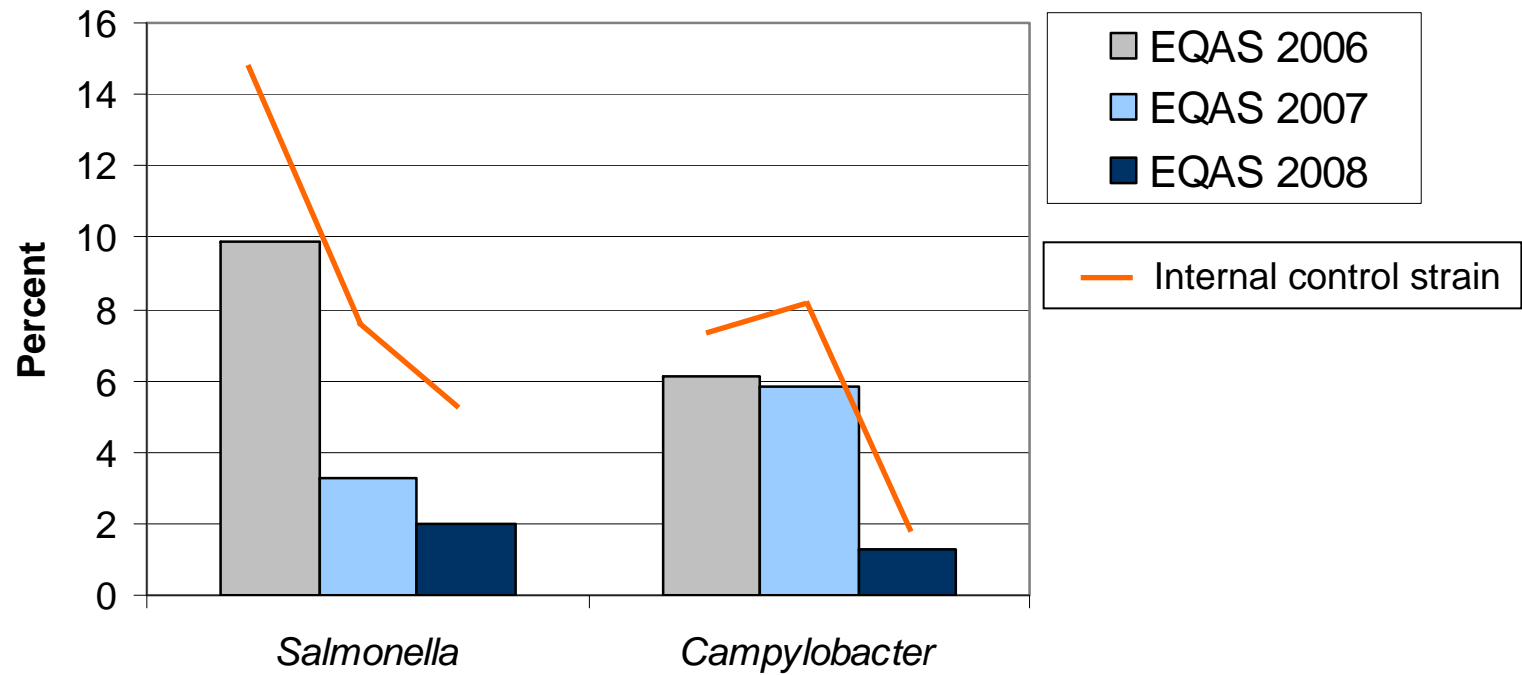
- Method
- Media, disk concentrations
- Reading of results
- Interpretation guidelines used
- Use of a QA-system (eg. QC strain)
- Routine
- ...

Methods and guidelines

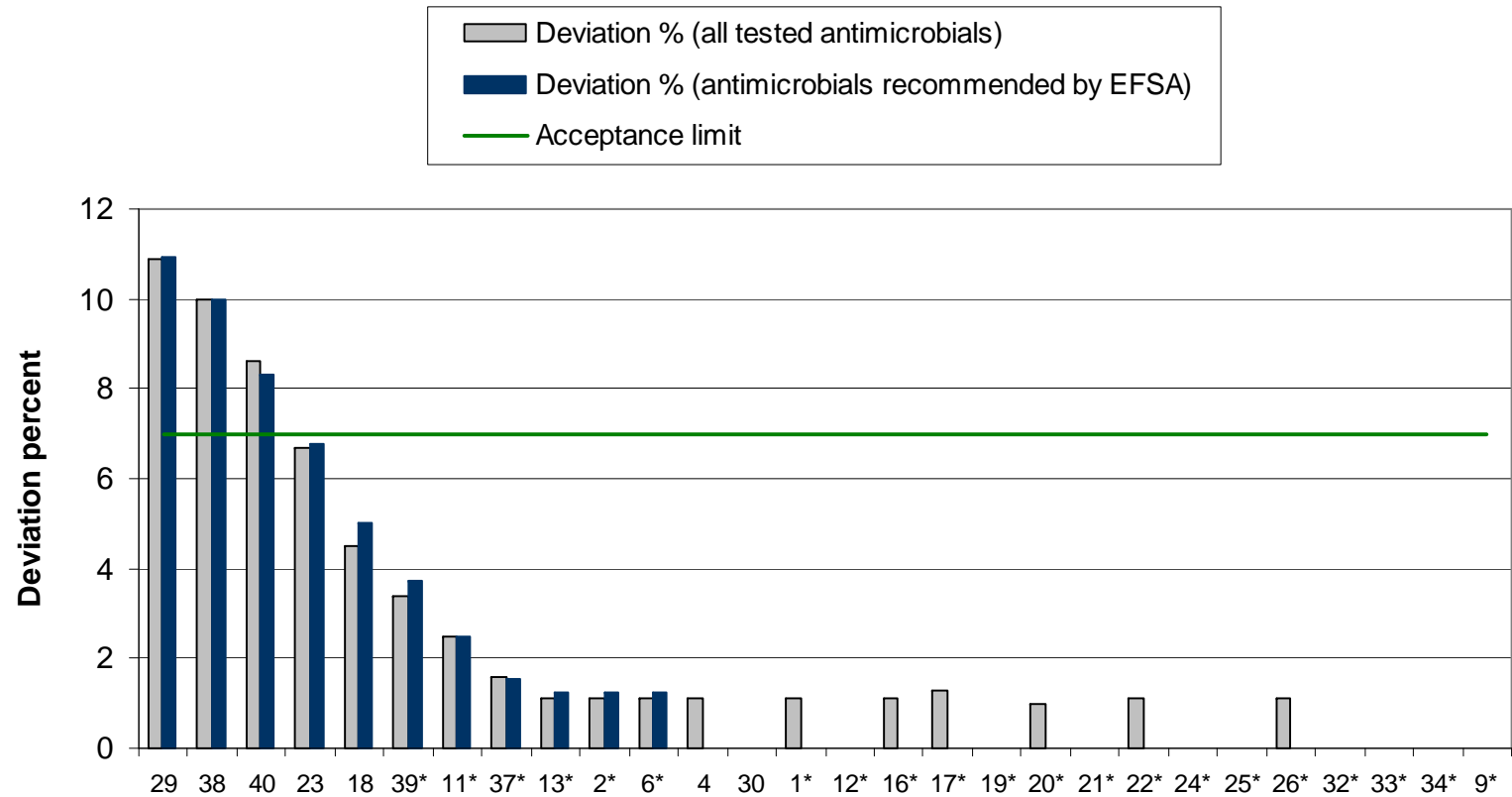
- MIC methods are recommended (For *Campylobacter*, reference to M45-A: an appearance of any zone of inhibition when performing DD would require MIC determination for accurate categorisation of susceptibility)
- Interpretation guidelines for MIC results are given in the protocol
- For interpretation of zone diameters (*Salmonella*), the laboratory's routine should be followed (however 'sensitive' and 'resistant' only, is accepted)



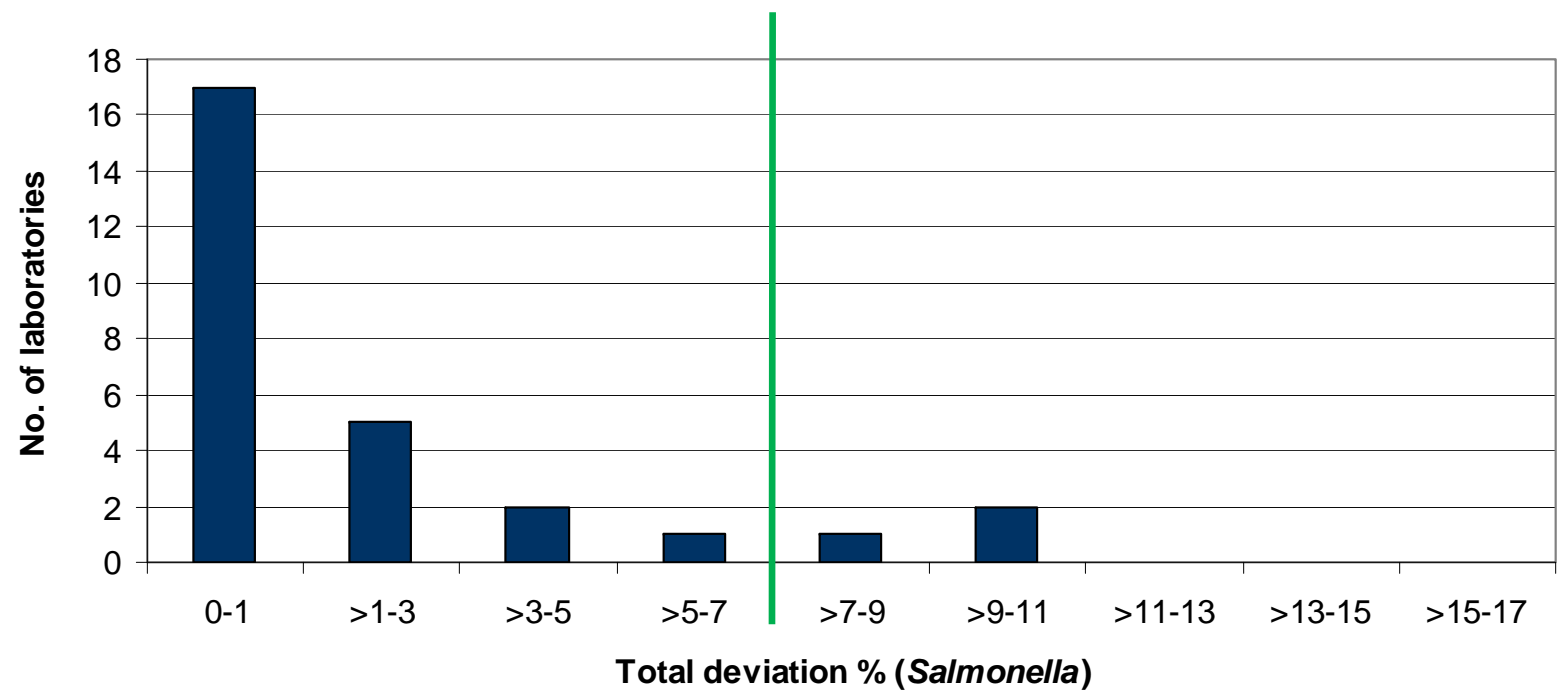
Comparison to former EQASs



Salmonella results – pr. lab



Salmonella results – intervals



Salmonella – deviations on antimicrobials

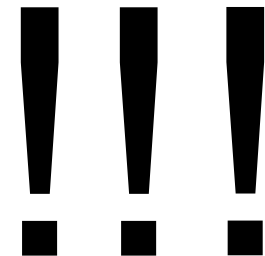
	% correct
Ampicillin, AMP	99.5
Cefotaxime, CTX	99.5
Ceftazidime, CAZ	94.7
Ceftiofur, XNL	100.0
Chloramphenicol, CHL	99.5
Ciprofloxacin, CIP	90.5
Gentamicin, GEN	98.6
Nalidixic acid, NAL	99.5
Streptomycin, STR	96.8
Sulphonamides, SMX	99.5
Tetracycline, TET	99.1
Trimethoprim, TMP	100.0

No CLSI guidelines for MIC-values, ARBAO-value is used

CAZ – towards *Salmonella*

- Results from the test strain/antimicrobial combination S3.3 and ceftazidime were only 60% correct
 - Strain was ESBL-producing:
 - Expected MIC for cefotaxime was >4 → RESISTANT
 - Expected MIC for ceftazidime was <0.5 → should be interpreted as RESISTANT due to resistance towards other cephalosporins

REMEMBER to regard all cephalosporins resistant if one cephalosporin shows resistance!



CIP – towards *Salmonella*

- Ciprofloxacin (9.5% deviation)
 - Low cut-off value is used (epidemiological cut off value is lower than the CLSI clinical breakpoint) => when performing DD the resistance is not seen
 - However, *Salmonella* resistant to nalidixic acid should also be interpreted as resistant to ciprofloxacin (protocol)
- Qnr-gene in S3.1
(75% correct for the S3.1/ciprofloxacin combination)
 - Nalidixic acid – sensitive
 - Ciprofloxacin – low level resistant

CIP – if performing DD

If you see reduced susceptibility towards ciprofloxacin

- Check the nalidixic acid result
 - ⇒ If this is resistant, ciprofloxacin should also be interpreted resistant (due to low epidemiological cut off value lower than the CLSI clinical breakpoint)
 - ⇒ If this is sensitive, you may want to check for a qnr-gene (e.g. by PCR)

ESBL-producing test strains – detection

- Percentage that reported the test strain ESBL-positive

	Strain S3.1 (CTX M-15 / SHV 12)	Strain S3.3 (CTX M-9)	Strain S3.5 (CTX M-15 like)
CTX, CAZ, XNL	6/6 (100%)	4/5 (80%)	5/6 (83%)
CTX, CAZ	11/12 (92%)	12/13 (92%)	13/13 (100%)
CTX, XNL	2/2 (100%)	2/2 (100%)	2/2 (100%)
CTX	2/2 (100%)	2/2 (100%)	2/2 (100%)

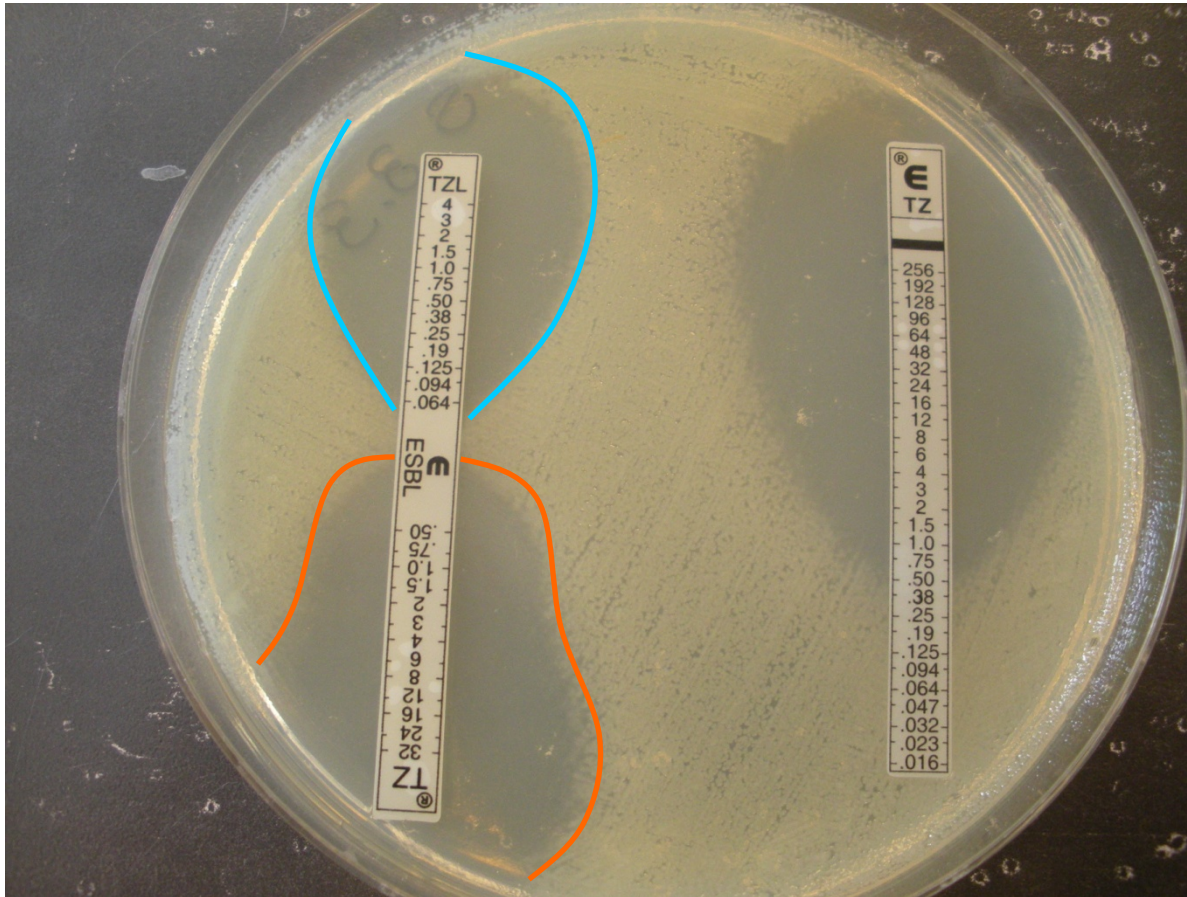
- All used combinations of CTX, CAZ and XNL were successful
- => however, some failed to regard all cephalosporins resistant if one cephalosporin showed resistance

ESBL-producing test strains – confirmation

	Strain S3.1 (CTX M-15 / SHV 12)	Strain S3.3 (CTX M-9)	Strain S3.5 (CTX M-15 like)
CTX/Ci:CTX	22/23 (96%)	20/22 (91%)	21/21 (100%)
CAZ/Ci:CAZ	22/23 (96%)	10/18 (56%)	22/23 (96%)
Confirmed ESBL	23/24 (96%)	22/24 (92%)	23/24 (96%)
FOX ^s	24/24 (100%)	24/24 (100%)	24/24 (100%)
AmpC not confirmed	23/24 (96%)	24/24 (100%)	23/24 (96%)

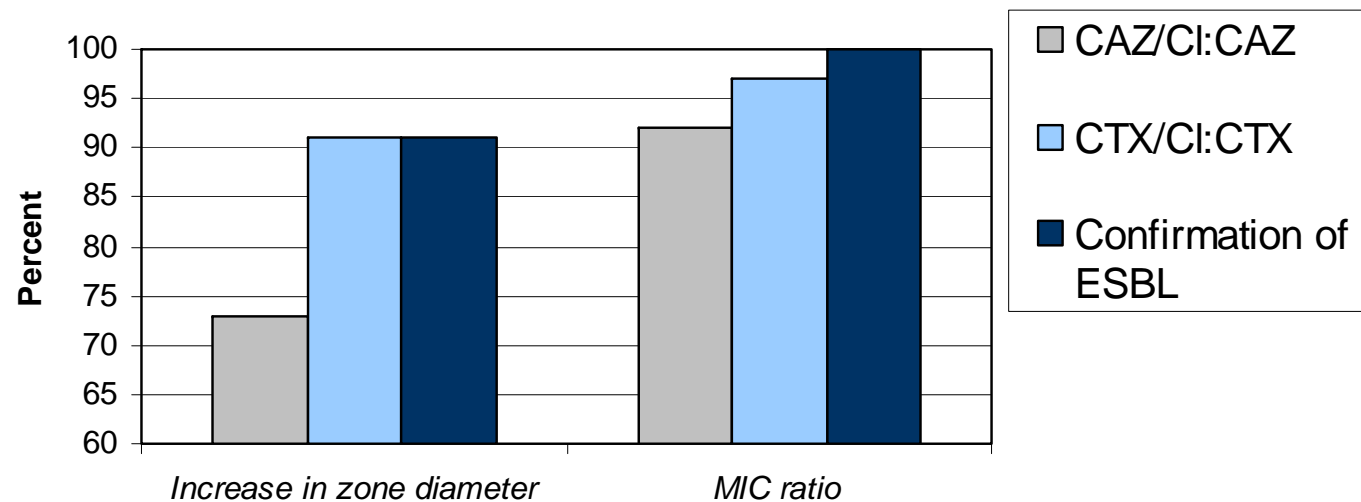
- Especially the result from the confirmatory test CAZ/Ci:CAZ deviated from the expected (however, only confirmed/not confirmed is evaluated)

S3.3 – CAZ/Cl:CAZ, confirmatory test



Confirmation of ESBL-production – methods

Results uploaded to the database (proportion in accordance with the expected):



ESBL-producing test strains – conclusions

ESBL-producing microorganisms is an emerging problem, and it should be high-priority for all NRL-AR's to be able to detect these problem strains, therefore:

- The NRLs decided on the WS 2008 that testing for ESBL-production is be mandatory in the EQASs

Remember:

- Strains resistant to one cephalosporin should be interpreted resistant to all cephalosporins – regardless of the value obtained

Campylobacter – deviations on antimicrobials

EQAS 2008	% correct
Chloramphenicol, CHL	100.0
Ciprofloxacin, CIP	97.5
Erythromycin, ERY	97.7*
Gentamicin, GEN	99.0
Nalidixic acid, NAL	96.4
Streptomycin, STR	98.4
Tetracycline, TET	96.4

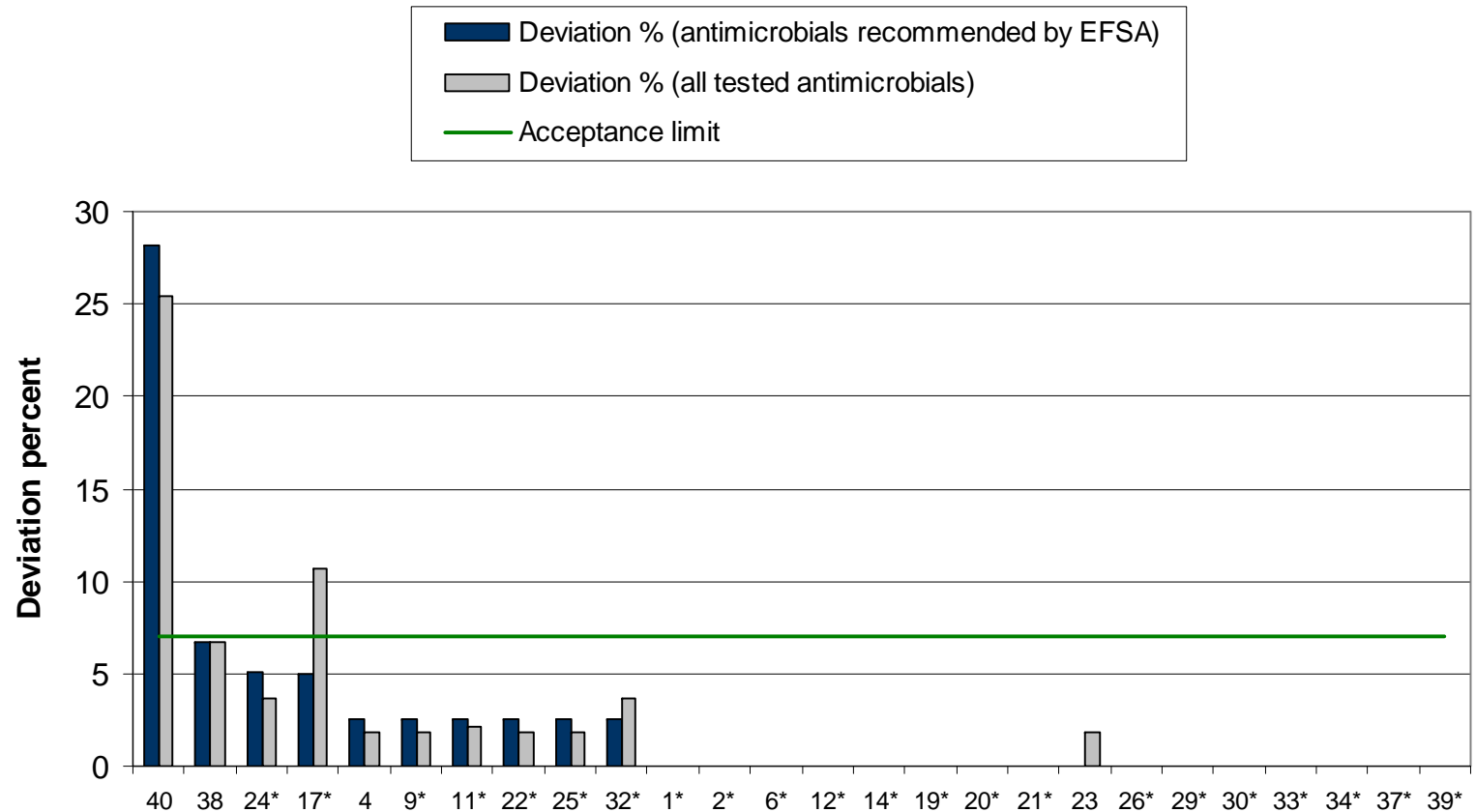
Majority of deviations are from disk diffusion tests

Originally 92.7%, however, some results were omitted

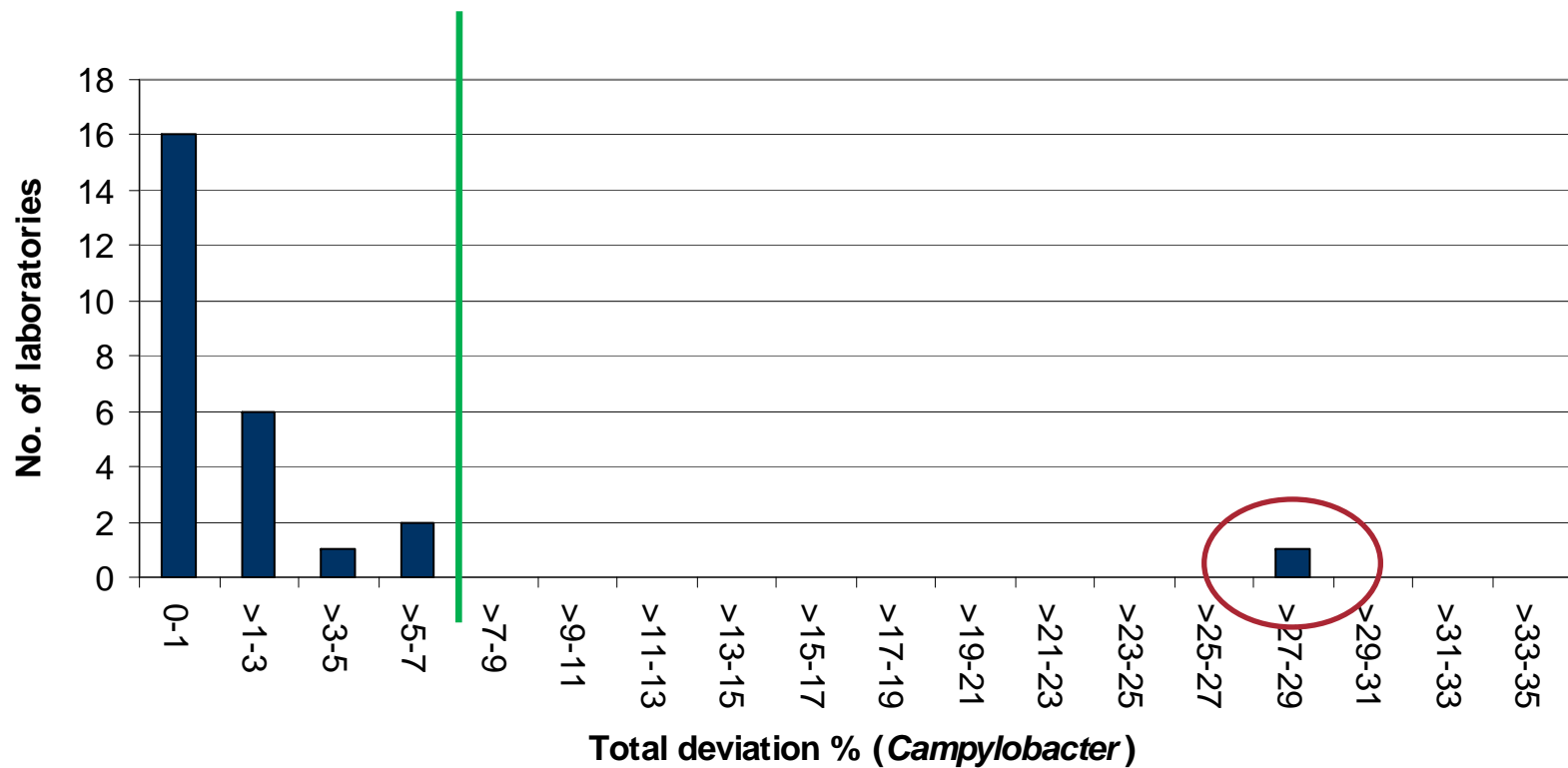
Omitted results, *Campylobacter*

- C3.6/erythromycin (72% correct)
 - 6/25 labs obtained an MIC of 1 $\mu\text{g/mL}$ or below \Rightarrow S
 - 1/25 labs obtained an interpretation as S by DD
 - Expected result was $>32 \mu\text{g/mL} \Rightarrow$ R
- \Rightarrow Results for this strain/antimicrobial combination omitted

Campylobacter results – pr. lab



Campylobacter results – intervals



Methods, *Campylobacter*

- Results obtained by disk diffusion on *Campylobacter* will not be included in future EQASs

QC strains – *Salmonella*, MIC

EQAS 2008 Antimicrobial	MIC determination <i>E. coli</i> ATCC 25922		
	Proportion of labs outside QC range	Obtained values in MIC steps (min/max)	
		Below lower QC limit	Above upper QC limit
Ampicillin, AMP	0/20 (0%)	-	-
Cefotaxime, CTX	0/21 (0%)	-	-
Cefoxitin, FOX	0/1 (0%)	-	-
Ceftazidime, CAZ	0/16 (0%)	-	-
Ceftiofur, XNL	0/3 (0%)	-	-
Chloramphenicol, CHL	0/20 (0%)	-	-
Ciprofloxacin, CIP	3/21 (14%)	-	1 step
Gentamicin, GEN	0/21 (0%)	-	-
Nalidixic acid, NAL	0/21 (0%)	-	-
Streptomycin, STR	0/21 (0%)	-	-
Sulphonamides, SMX	0/14 (0%)	-	-
Tetracycline, TET	0/21 (0%)	-	-
Trimethoprim, TMP	0/21 (0%)	-	-

QC strains – *Salmonella*, disc diffusion

EQAS 2008 Antimicrobial	Disk diffusion <i>E. coli</i> ATCC 25922		
	Proportion of labs outside QC range	Obtained values in mm zones (min/max)	
		Below lower QC limit	Above upper QC limit
Ampicillin, AMP	0/6 (0%)	-	-
Cefotaxime, CTX	0/4 (0%)	-	-
Cefoxitin, FOX	0/5 (0%)	-	-
Ceftazidime, CAZ	0/4 (0%)	-	-
Ceftiofur, XNL	0/3 (0%)	-	-
Chloramphenicol, CHL	0/6 (0%)	-	-
Ciprofloxacin, CIP	0/6 (0%)	-	-
Gentamicin, GEN	0/6 (0%)	-	-
Imipenem, IMI	0/4 (0%)	-	-
Nalidixic acid, NAL	0/6 (0%)	-	-
Streptomycin, STR	0/6 (0%)	-	-
Sulphonamides, SMX	1/3 (33%)	1	-
Tetracycline, TET	0/6 (0%)	-	-
Trimethoprim, TMP	0/5 (0%)	-	-

QC strains – *Campylobacter*, MIC

EQAS 2008 Antimicrobial	MIC determination <i>C. jejuni</i> ATCC 33560		
	Proportion of labs outside QC range	Obtained values in MIC steps (min/max)	
		Below lower QC limit	Above upper QC limit
Chloramphenicol, CHL	1/16 (6%)	1 step	-
Ciprofloxacin, CIP	1/22 (4%)	-	1 step
Erythromycin, ERY	4/22 (18%)	2 steps	1 step
Gentamicin, GEN	1/20 (5%)	1 step	-
Nalidixic acid, NAL	3/20 (15%)	3 steps	-
Tetracycline, TET	3/20 (15%)	1 step	2 steps

- All labs performing MIC on *Campy*, uploaded QC-strain data
- The proportion within the QC intervals in 2007: 83.8%
- The proportion within the QC intervals in 2008: 89.2%
- Two laboratories each had four of the 13 deviations

Summing up

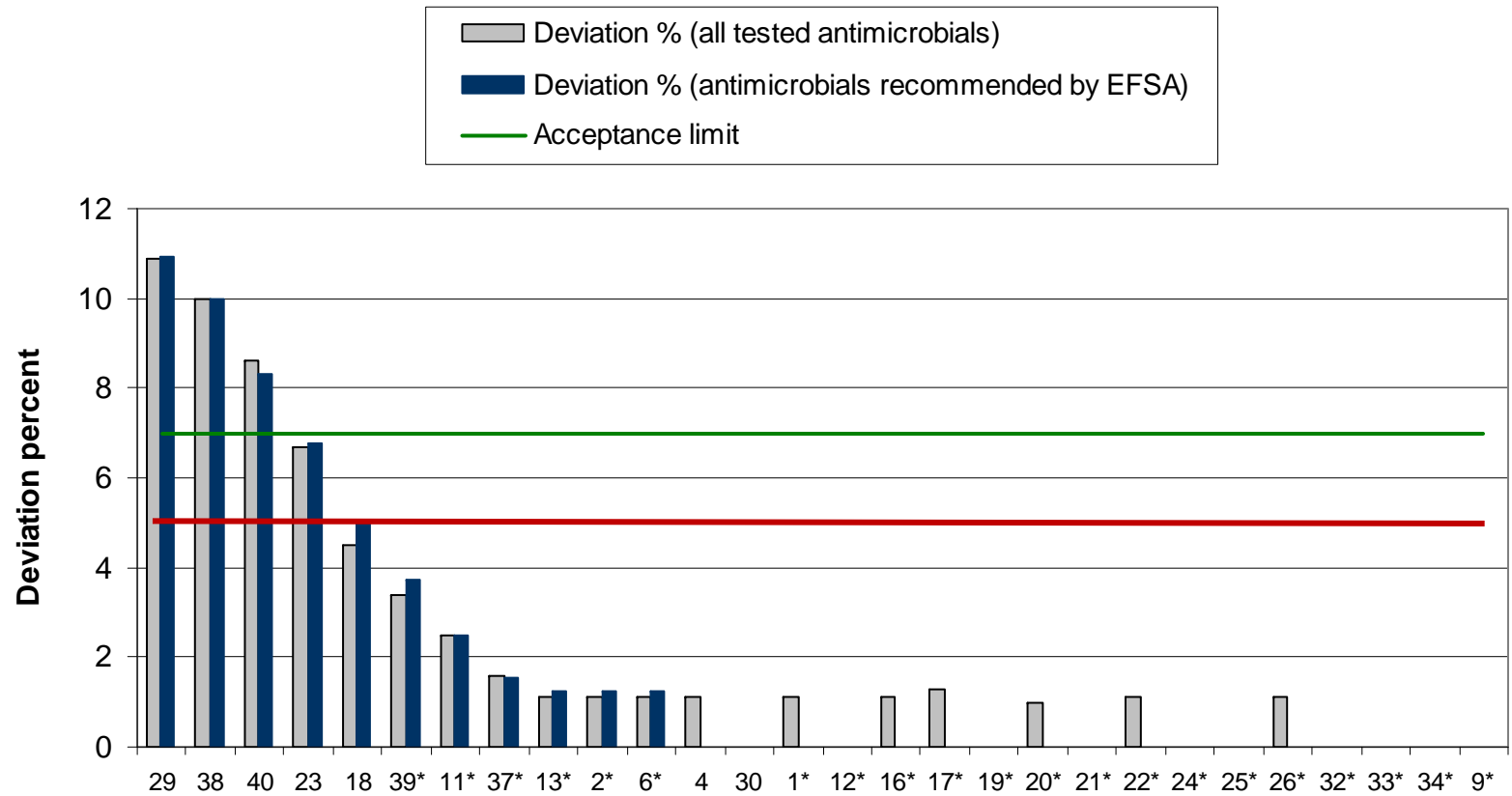
- 27 labs participated in the *Salmonella* trial – 24 labs performed with deviation levels below the acceptance level, of which 17 labs had no deviations
- 26 labs participated in the *Campylobacter* trial – 25 labs performed with deviation levels below the acceptance level, of which 16 labs had no deviations

The lab with a high deviation percent (*Campy*)

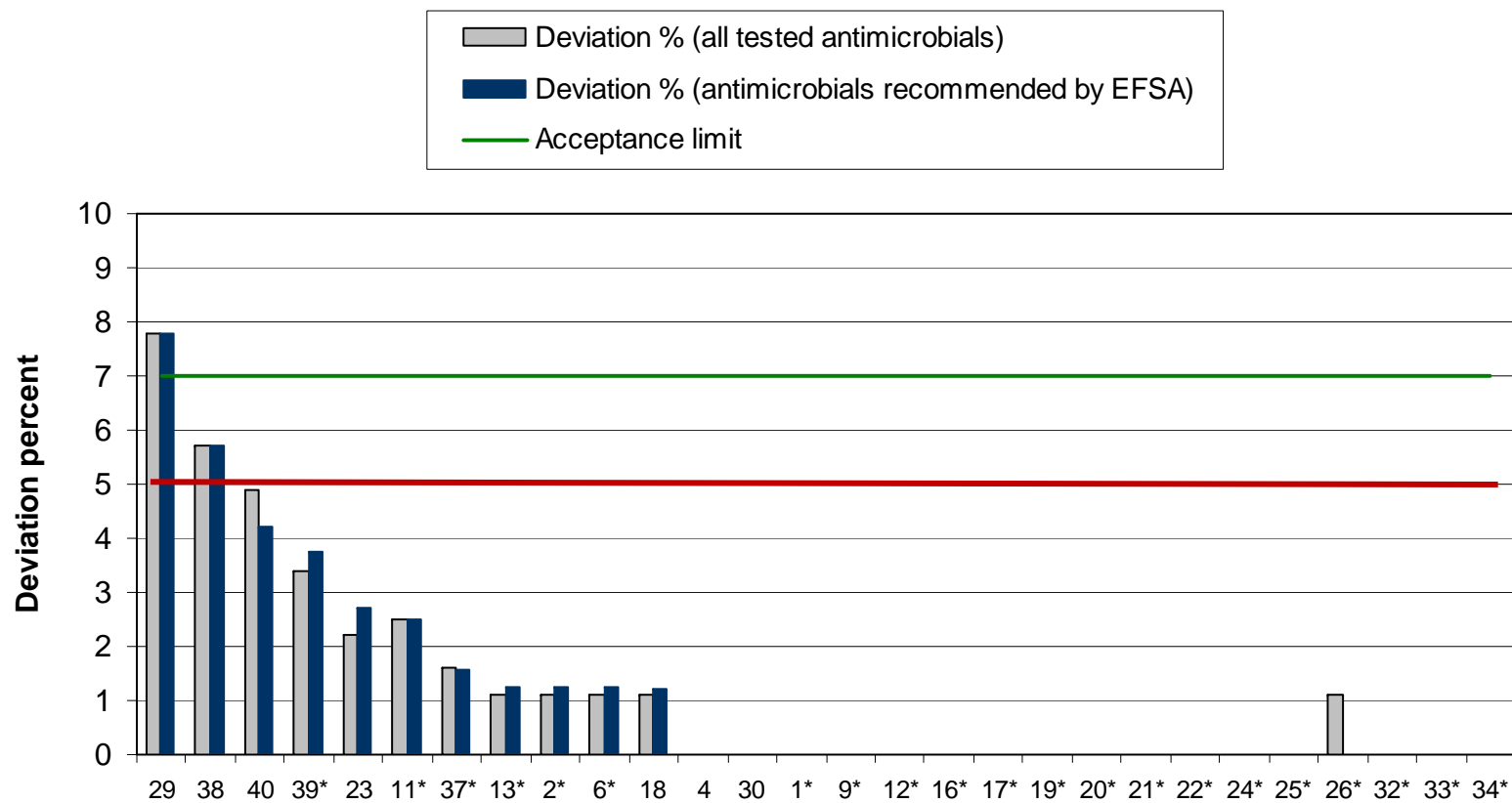
- Has received training (March 2009)



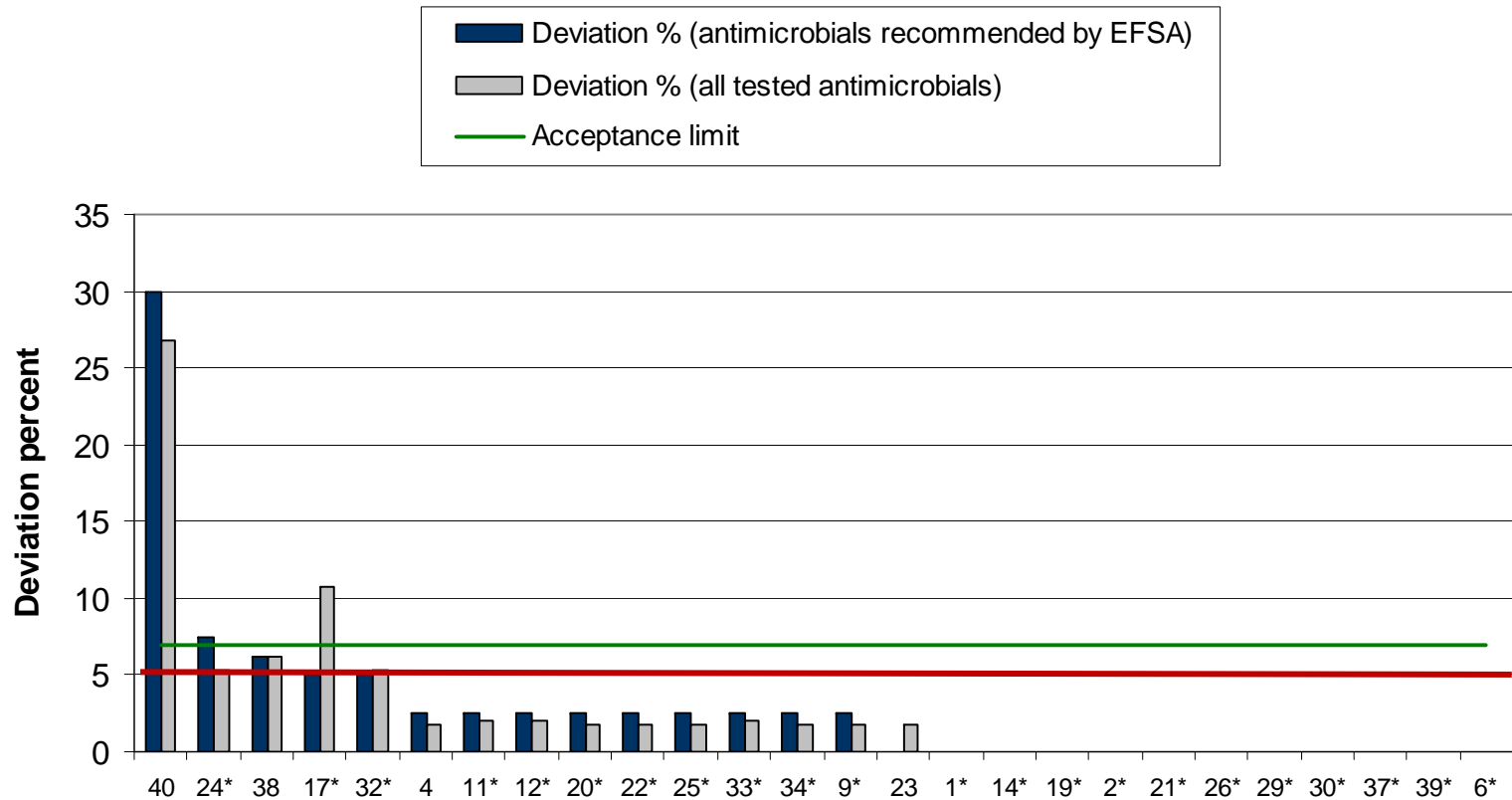
Salmonella, new goal?



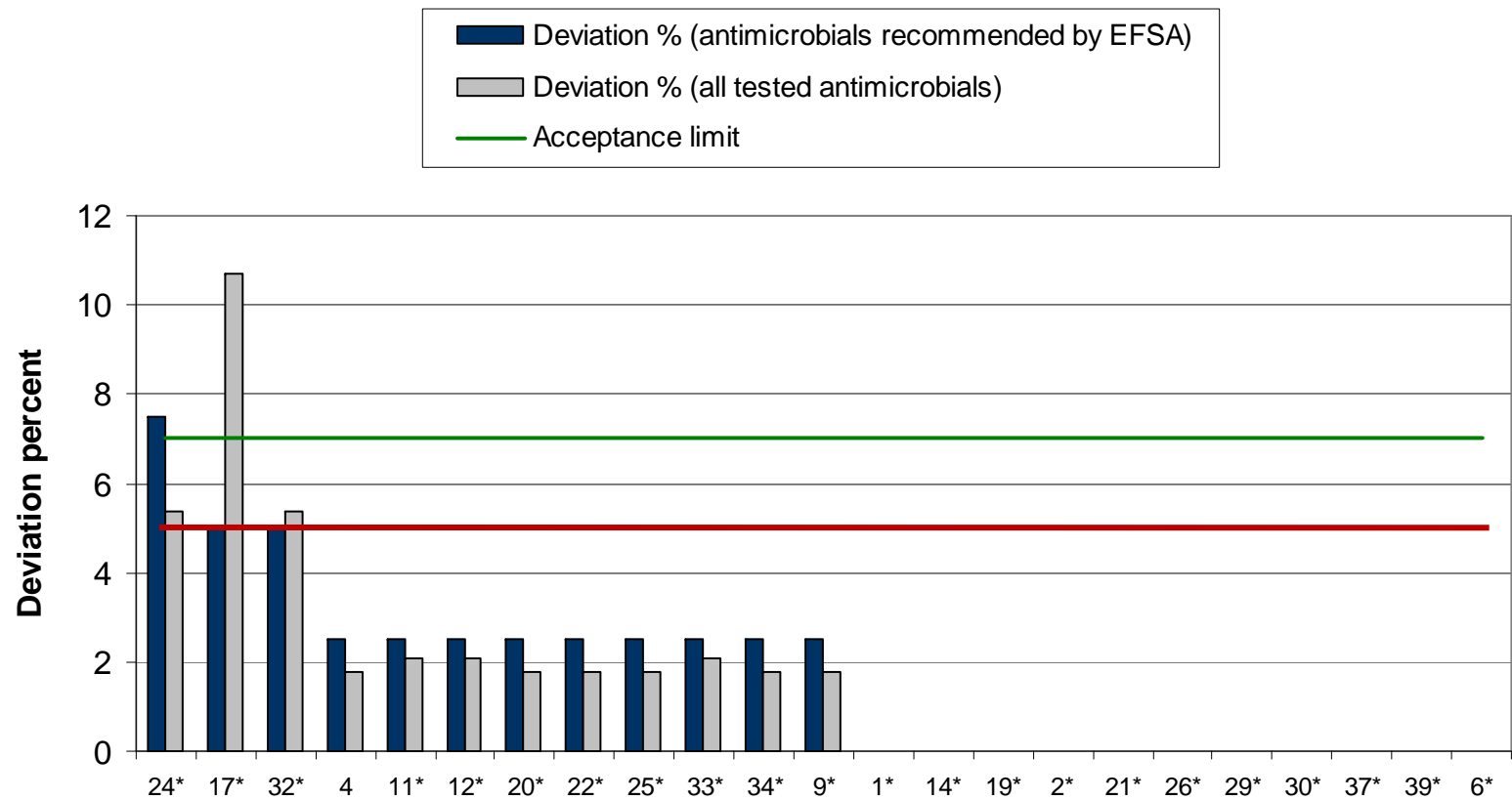
Salmonella – cip and ceph deviations omitted



Campylobacter, new goal?



Campylobacter – DD-results omitted



Thanks for your attention!

(Additional) questions and/or items for discussion?

