



DTU Food
National Food Institute

EQAS 2007

Salmonella and Campylobacter

CRL workshop, June 19-20, 2008

Susanne Karlsrose (suska@food.dtu.dk)

Main objectives of the CRL EQAS's

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

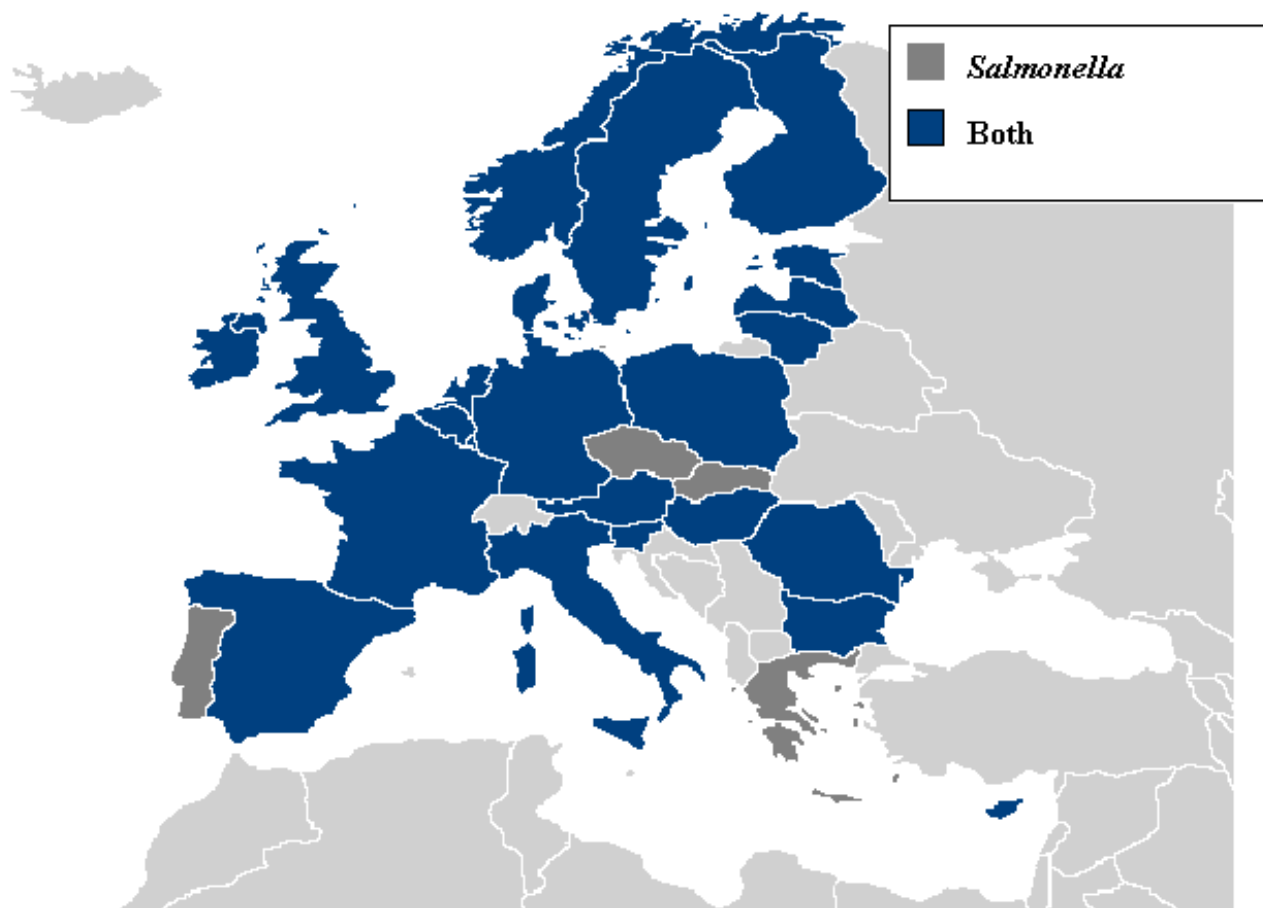


Outline of Salm/Camp EQAS, 2007

- 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



Participation in the Salm/Camp EQAS, 2007



Issues important for differences in performance

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

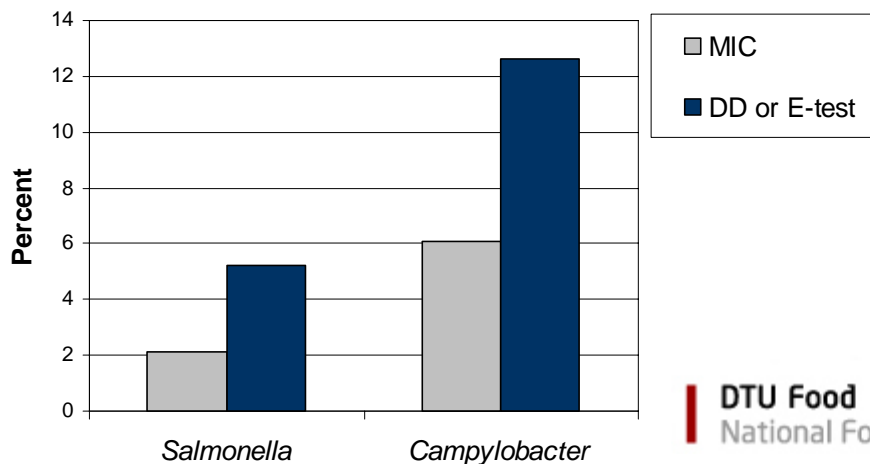
Further investigation of a laboratory's deviations includes looking into:

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

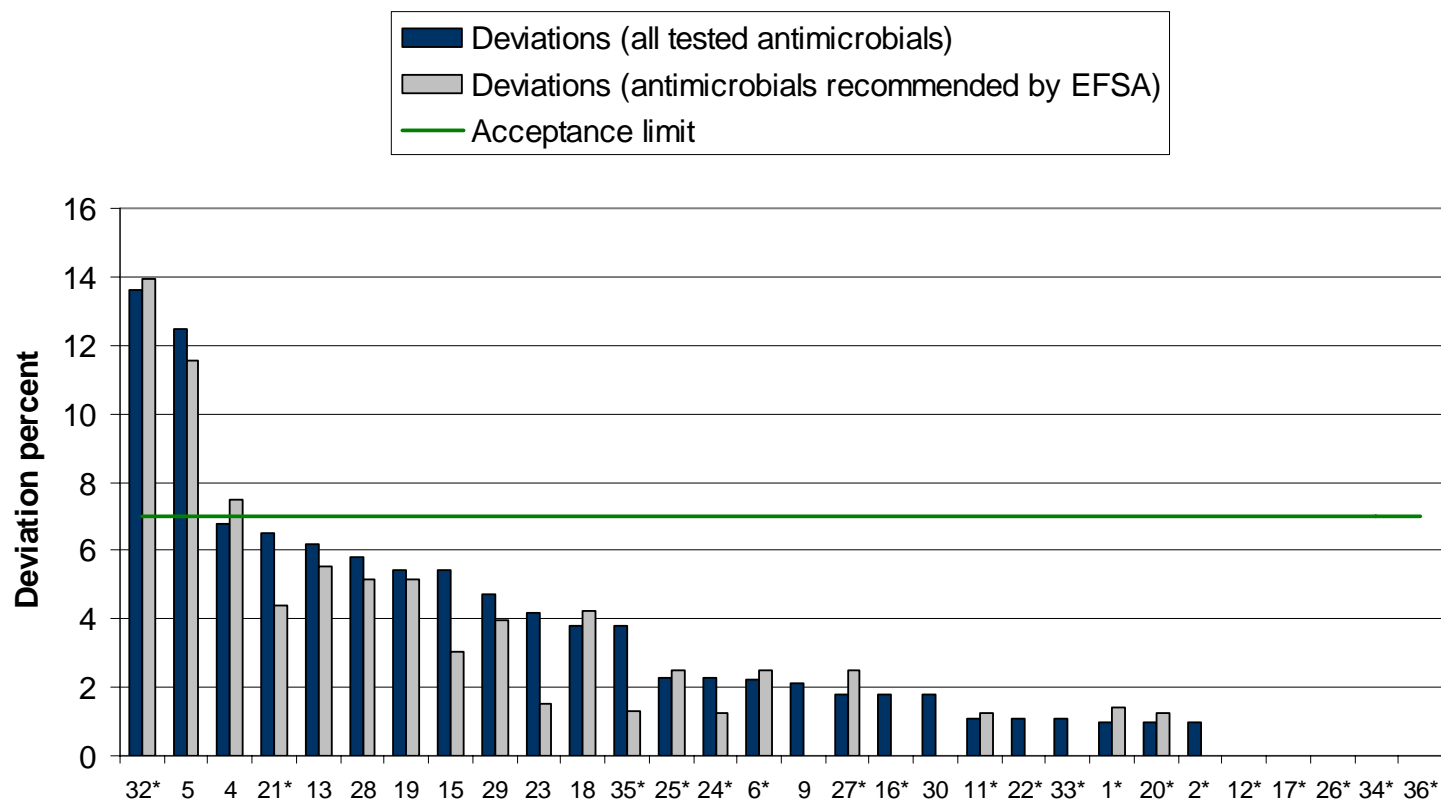


Methods and guidelines

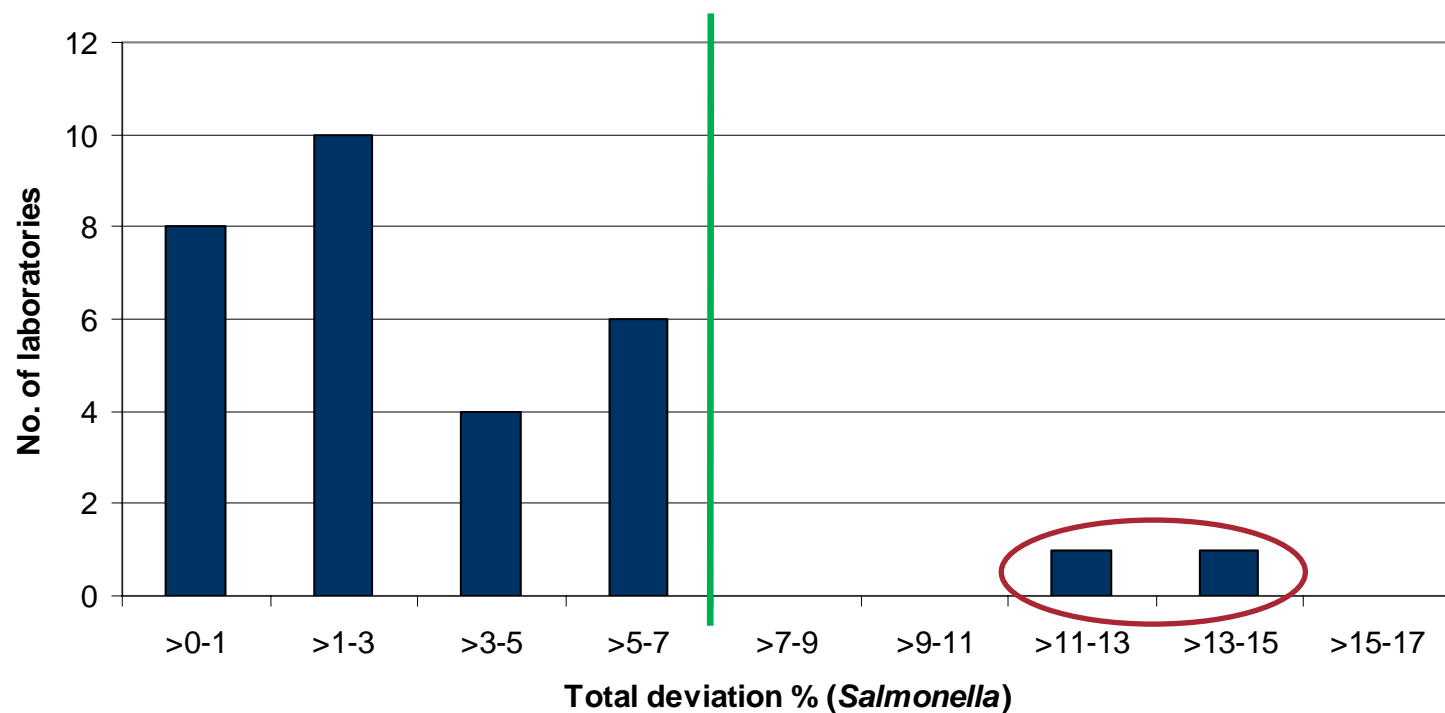
- MIC methods are recommended (For *Campylobacter*, reference to M45-A: an appearance of any zone of inhibition would require MIC determination for accurate categorisation of susceptibility)
- Interpretation guidelines for MIC results are given in the protocol
- For interpretation of zone diameters (*Salmonella*), the laboratory's routine should be followed (however 'intermediary' is not accepted)



Salmonella results – pr. lab



Salmonella results – intervals



Tricky *Salmonella* test strains

- Results from test strain S2.2 in total deviated 7.7%
 - Strain was ESBL-producing, and the cephalosporin results caused some problems
 - Also, amoxicillin/clavulanic acid and ciprofloxacin caused problems for a number of participants
 - Same strain deviated 14.7% in the 2006 trial

No plasmid mediated quinolone resistance...

- Test strain S2.4 carried a plasmid with a *qnrS*-gene when first tested and verified. The plasmid seemed to be lost before tested by the NRL's (MIC changed from 0.5 µg/mL to 0.03 µg/mL). Expected result was corrected in the database

Tricky antimicrobials (*Salmonella*)

- Amoxicillin/clavulanic acid (18.5% deviation)
 - Expected MIC values were close to cut off values (borderline)
 - Same antimicrobial deviated 30% in the 2006 trial
- Ciprofloxacin (10% deviation)
 - Majority of cip-deviations: Disk diffusion (epidemiological cut off value is lower than the CLSI clinical breakpoint)
 - NB: *Salmonella* resistant to nalidixic acid should also be interpreted as resistant to ciprofloxacin (protocol)
 - Same antimicrobial deviated 20.2% in the 2006 trial

ESBL-producing test strains – detection I

	Strain S2.2 (CTX M-9)		Strain S2.8 (CMY-2)	
	ESBL/AmpC NOT indicated	ESBL/AmpC indicated	ESBL/AmpC NOT indicated	ESBL/AmpC indicated
CTX, CAZ, XNL	1 of 6 (17%)	5 of 6 (83%)	0	7 of 7 (100%)
CTX, CAZ	5 of 10 (50%)	5 of 10 (50%)	1 of 9 (11%)	8 of 9 (89%)
CTX, XNL	0	6 of 6 (100%)	0	6 of 6 (100%)
CAZ	2 of 2 (100%)	0	0	1 of 1 (100%)
CTX	2 of 3 (67%)	1 of 3 (33%)	1 of 2 (50%)	1 of 2 (50%)
XNL	0	2 of 2 (100%)	0	2 of 2 (100%)

- The sole use of CTX or CAZ did not successfully indicate the CTX M-9 gene
 - Also, the combination CTX and CAZ seems unsuccessful, however, only in one case both were found sensitive
- => remember: if one cephalosporin shows resistance, all cephalosporins should be regarded resistant

ESBL-producing test strains – detection II

	Strain S2.2 (CTX M-9)		Strain S2.8 (CMY-2)	
	ESBL/AmpC NOT indicated	ESBL/AmpC indicated	ESBL/AmpC NOT indicated	ESBL/AmpC indicated
CTX, CAZ, XNL	1 of 6 (17%)	5 of 6 (83%)	0	7 of 7 (100%)
CTX, CAZ	5 of 10 (50%)	5 of 10 (50%)	1 of 9 (11%)	8 of 9 (89%)
CTX, XNL	0	6 of 6 (100%)	0	6 of 6 (100%)
CAZ	2 of 2 (100%)	0	0	1 of 1 (100%)
CTX	2 of 3 (67%)	1 of 3 (33%)	1 of 2 (50%)	1 of 2 (50%)
XNL	0	2 of 2 (100%)	0	2 of 2 (100%)

- Combining CTX and XNL, as well as CTX, CAZ and XNL was successful
- The use of ceftiofur as the sole cephalosporin was also effective

ESBL-producing test strains – confirmation

	Strain S2.2 (CTX M-9)		Strain S2.8 (CMY-2)	
	NOT confirmed	Confirmed	NOT confirmed	Confirmed
CTX/Ci:CTX	1 of 23 (4%)	22 of 23 (96%)	12 of 14 (86%)	2 of 14 (14%)
CAZ/Ci:CAZ	12 of 20 (60%)	8 of 20 (40%)	12 of 14 (86%)	2 of 14 (14%)
FOX	16 of 17 (94%)	1 of 17 (6%)	1 of 17 (6%)	16 of 17 (94%)
Confirmed ESBL in the database	1 of 23 (4%)	22 of 23 (96%)	15 of 15 (100%)	0
Confirmed AmpC in the database	16 of 16 (100%)	0	3 of 18 (17%)	15 of 18 (83%)

??

- The 'true ESBL' (S2.2) was confirmed by 96%
- The ampC was had a lower confirmation percentage, however...

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:

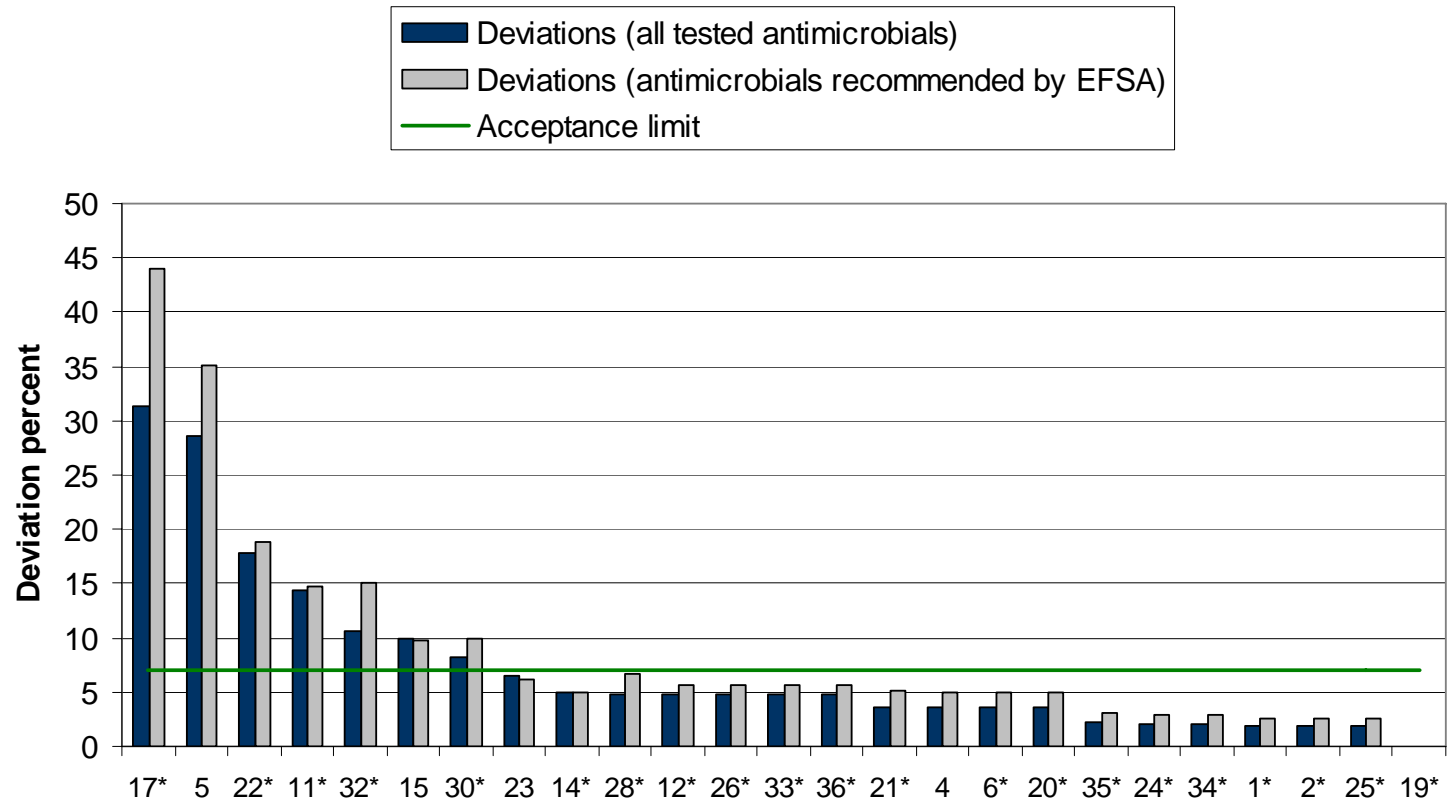
- As of the EC/Ent/Staph 2008 – testing for ESBL-production will be mandatory

Remember:

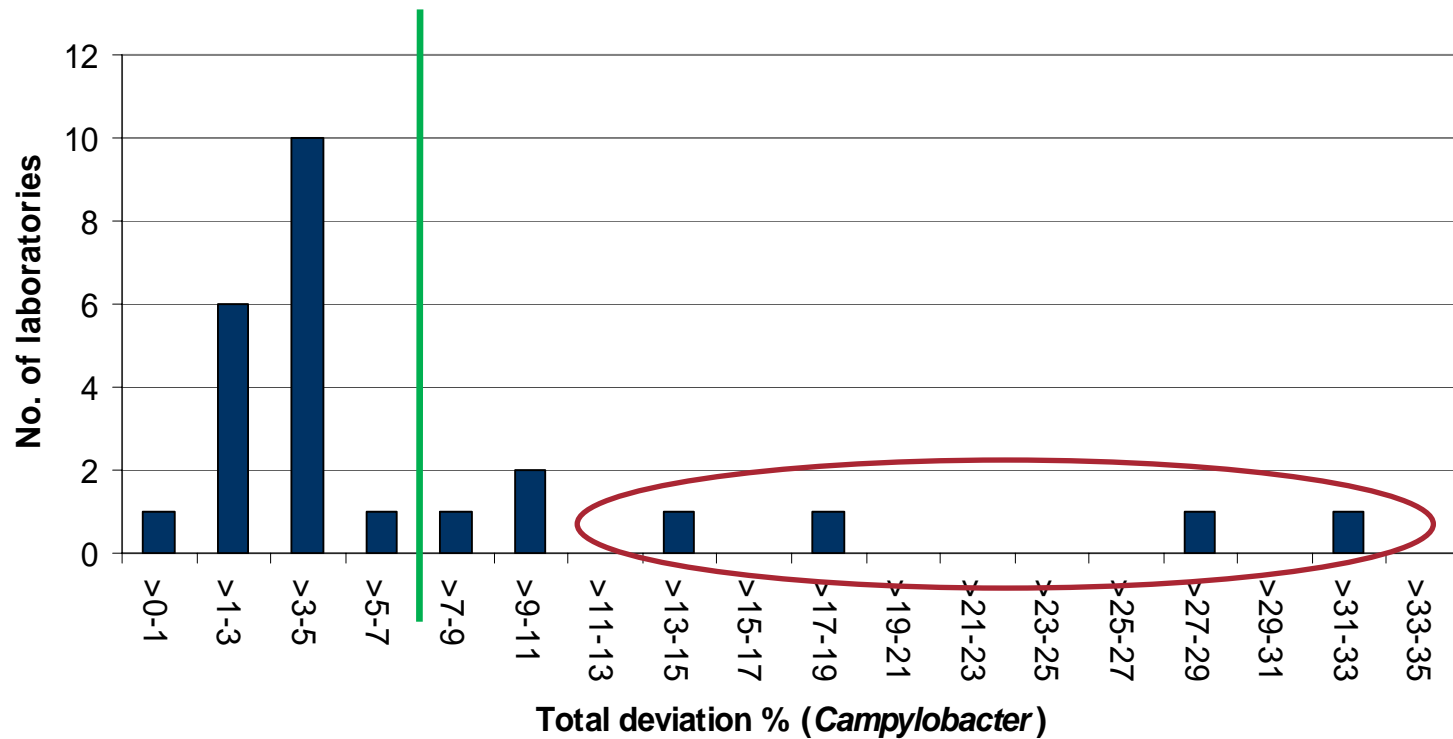
- Test strains resistant to one cephalosporin should be interpreted resistant to all cephalosporins – regardless of the value obtained



Campylobacter results – pr. lab



Campylobacter results – intervals



Difficult *Campy* test strains and antimicrobials

- Results from test strain C2.1 in total deviated 14.3%
 - 18 NRL's found this strain resistant to streptomycin, whereas the expected interpretation was S
- Results from test strain C2.5 in total deviated 15.6%
 - 17 NRL's found this strain sensitive to tetracycline, whereas the expected interpretation was R
- Results from test strains C2.6 and C2.7 also deviated more than acceptable (10.3% and 8.2%). No obvious general reason
- Streptomycin (12.6% deviation)
- Tetracycline (9.9% deviation)



Difficult *Campy* test strains – C2.1, streptomycin

- Switched somehow??

	CHL	CIP	ERY	GEN	NAL	STR	TET
2.1	S	S	S	S	S	S	S
	≤ 2	0.125	≤ 0.5	0.25	4	≤ 2	≤ 0.25
2.2	S	S	S	S	S	R	S
	2	0.125	≤ 0.5	0.5	4	>16	0.5
Retest	S	S	S	S	S	R	S
	4	0.25	2-4	0.5	8	>16	1

- Let's look at the results – and talk QC!

Difficult *Campy* test strains – C2.1, streptomycin

Interp	Value	Exp Interp	Exp MIC
Test strain C-2.1, Streptomycin			
R	32	S	≤ 2
R	64	S	≤ 2
R	64	S	≤ 2
R	64	S	≤ 2
R	>64	S	≤ 2
R	128	S	≤ 2
R	>64	S	≤ 2
R	64	S	≤ 2
R	=32	S	≤ 2
R	≥ 4	S	≤ 2
R	>64	S	≤ 2
R	6	S	≤ 2
R	>16	S	≤ 2
R	64	S	≤ 2
R	>64	S	≤ 2
R	64	S	≤ 2
R	6	S	≤ 2
R	32	S	≤ 2
S	1	S	≤ 2
S	≤ 1	S	≤ 2
S	≤ 0.5	S	≤ 2

Interp	Value	Exp Interp	Exp MIC
Test strain C-2.2, Streptomycin			
R	>16	R	>16
R	>64	R	>16
R	64	R	>16
R	64	R	>16
R	>64	R	>16
R	64	R	>16
R	64	R	>16
R	=32	R	>16
R	≥ 8	R	>16
R	>64	R	>16
R	6	R	>16
R	>16	R	>16
R	>32	R	>16
R	32	R	>16
R	32	R	>16
R	64	R	>16
R	64	R	>16
R	6	R	>16
R	>64	R	>16
R	64	R	>16
R	>64	R	>16

Difficult *Campy* test strains – C2.5, tetracycline

Obtained	Value	Expected	Exp MIC	Mark	Method
S	<=0.12	R	4	0	MIC
S	<=1	R	4	0	AGA
S	=2	R	4	0	MIC
S	0.5	R	4	0	MIC
S	0.5	R	4	0	MIC
S	0.75	R	4	0	ET
S	1	R	4	0	MIC
S	1	R	4	0	ET
S	1	R	4	0	MIC
S	1	R	4	0	MIC
S	1	R	4	0	MIC
S	1	R	4	0	MIC
S	2	R	4	0	MIC
S	2	R	4	0	MIC
S	2	R	4	0	MIC
S	2	R	4	0	MIC
S	2	R	4	0	MIC
S	2	R	4	0	MIC
S	26	R	4	0	DD
R	4	R	4	1	MIC
R	4	R	4	1	MIC
R	4	R	4	1	MIC
R	4	R	4	1	MIC
R	4	R	4	1	MIC

- Test strain was tested, verified and retested
- Borderline
(expected MIC: 4µg/mL and cut off: R>2)

QC strains – *Salmonella*, MIC

EQAS 2007 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
Amoxicillin cl., AUG	0/4 (0%)	-	-
Ampicillin, AMP	0/19 (0%)	-	-
Cefotaxime, CTX	0/16 (0%)	-	-
Cefoxitin, FOX	0/3 (0%)	-	-
Cefpodoxime, POD	0/1 (0%)	-	-
Ceftazidime, CAZ	0/9 (0%)	-	-
Ceftiofur, XNL	0/9 (0%)	-	-
Chloramphenicol, CHL	1/18 (6%)	-	1 step
Ciprofloxacin, CIP	6/18 (33%)	-	2 steps
Gentamicin, GEN	0/19 (0%)	-	-
Imipenem, IMI	0/0 (0%)	-	-
Nalidixic acid, NAL	0/19 (0%)	-	-
Streptomycin, STR	0/17 (0%)	-	-
Sulphonamides, SMX	1/16 (6%)	-	5 steps
Tetracycline, TET	1/19 (5%)	-	1 step
Trimethoprim, TMP	0/19 (0%)	-	-

SMX is bacteriostatic,
not bactericidal...
Reading error?

QC strains – *Salmonella*, disc diffusion

EQAS 2007 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
Amoxicillin cl., AUG	1/9 (11%)	-	2
Ampicillin, AMP	2/8 (25%)	2	1
Cefotaxime, CTX	2/11 (18%)	5	2
Cefoxitin, FOX	1/3 (33%)	-	3
Cefpodoxime, POD	0/2 (0%)	-	-
Ceftazidime, CAZ	1/8 (12.5%)	-	2
Ceftiofur, XNL	1/4 (25%)	-	1
Chloramphenicol, CHL	2/10 (20%)	-	3
Ciprofloxacin, CIP	0/9 (0%)	-	-
Gentamicin, GEN	0/10 (0%)	-	-
Imipenem, IMI	2/2 (100%)	-	6
Nalidixic acid, NAL	0/10 (0%)	-	-
Sulphonamides, SMX	1/9 (11%)	-	7
Tetracycline, TET	1/10 (10%)	-	1
Trimethoprim, TMP	0/7 (0%)	-	-

QC strains – *Campylobacter*, MIC

EQAS 2007 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	0/10 (0%)	-	-
Ciprofloxacin, CIP	5/21 (24%)	-	1 step
Doxycycline, DOX	-	-	-
Erythromycin, ERY	3/21 (14%)	-	1 step
Gentamicin, GEN	2/13 (15%)	1 step	2 steps
Meropenem, MERO	-	-	-
Nalidixic acid, NAL	0/19 (0%)	-	-
Tetracycline, TET	1/19 (5%)	-	1 step

Erythromycin improved from 39% in the 2006 trial

QC strains – conclusions

- All laboratories participating in the *Salmonella* EQAS performed QC testing of the reference strain
 - For MIC, ciprofloxacin had a high deviation percent
- For *Campylobacter*, two laboratories did not perform QC testing of the reference strain (one used DD and the other microbroth dilution)
 - Ciprofloxacin had a high deviation percent



Summing up

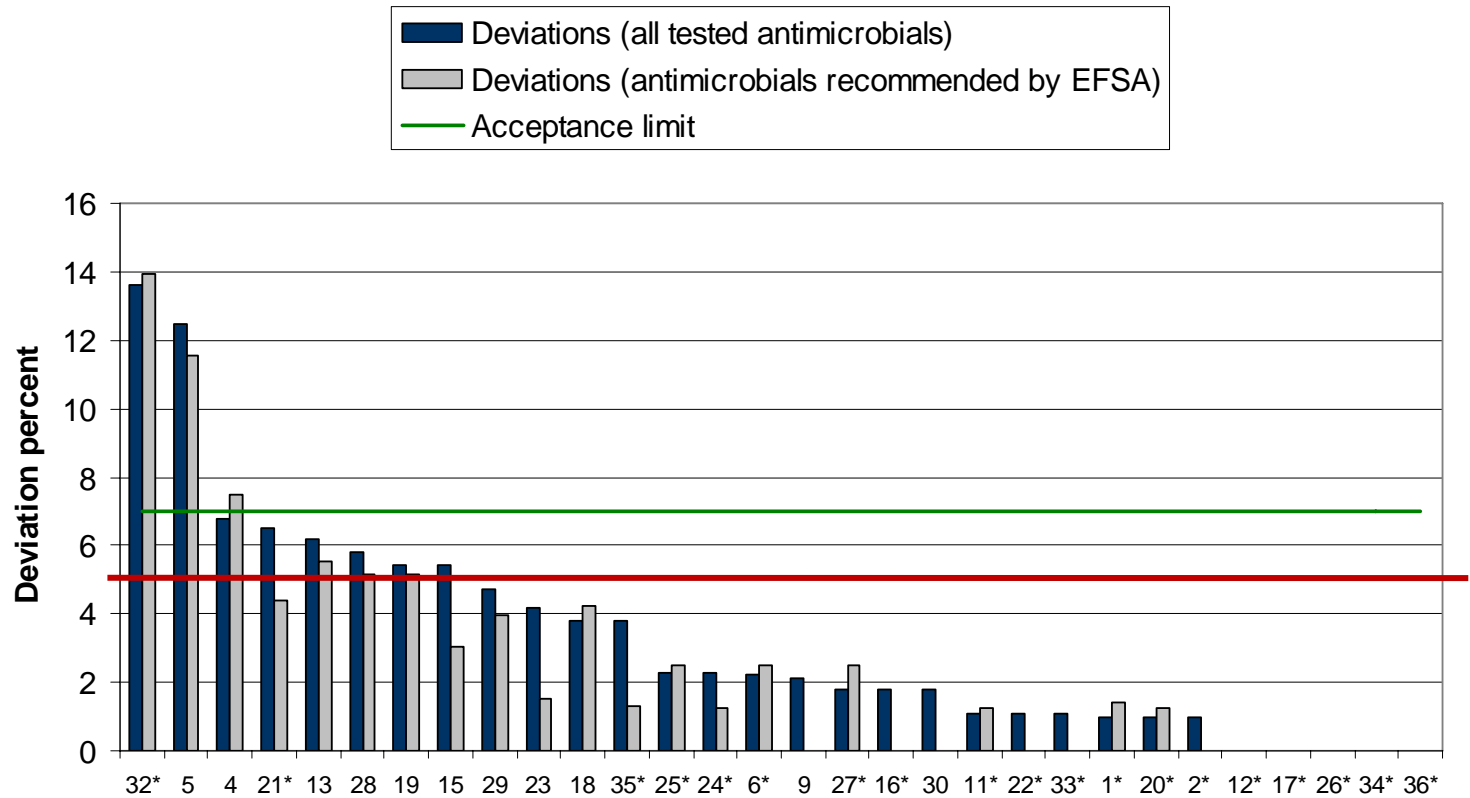
- 30 labs participated in the *Salmonella* trial – 28 labs performed well, with 5 labs having no deviations
- 25 labs participated in the *Campylobacter* trial – 18 labs performed well, with 1 lab having no deviations

Labs with a high deviation percent

- Are expected to participate in investigating the reasons behind the unsatisfactory performance
- Have received retests
- Will be considered when inviting for training course 2009



Salmonella, new goal?



Campylobacter, new goal?

