



EQAS 2011

Salmonella, Campylobacter
and optional genotypic characterisation
EURL-AR workshop, April 23-24th, 2012

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Outline of Salm/Camp EQAS 2011

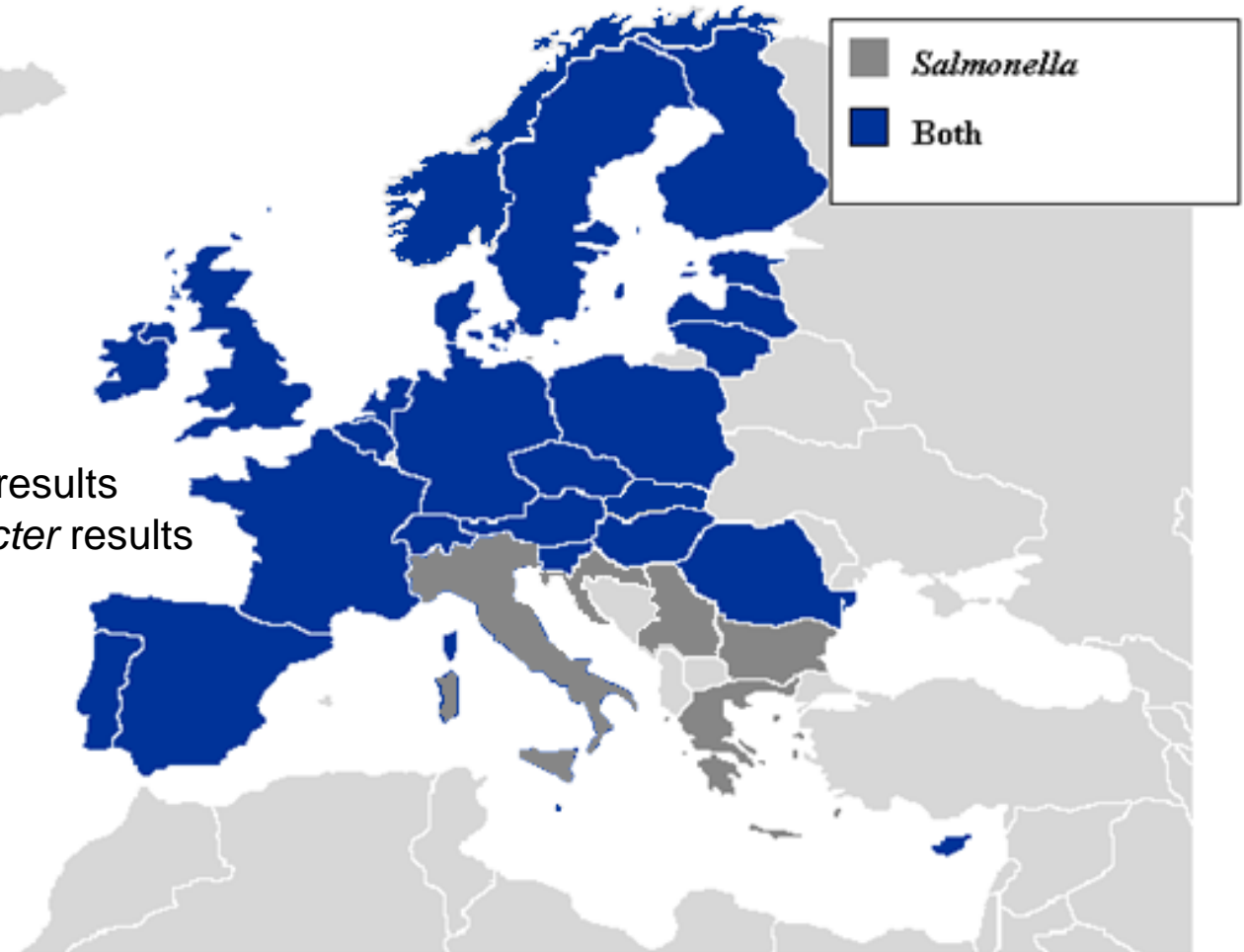
- AST of eight *Salmonella* and eight *Campylobacter*
- New participants were supplied with original reference strains ATCC 25922 and ATCC 33560 for QC testing
- Individual database-generated evaluation report
- Overall comparison and evaluation of all results
- Aim: That all NRL-AR's perform AST with less than 5% incorrect interpretations
- Optional: genotypic characterization of a MDR *Salmonella* strain and *S. aureus*



Participation

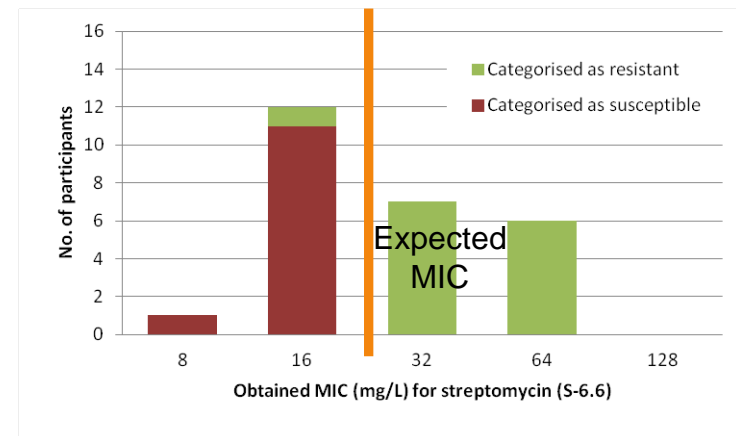
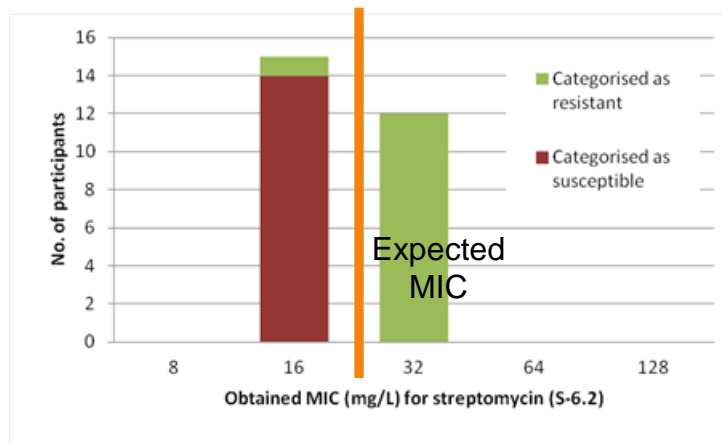
30 countries

- 34 sets of *Salmonella* results
- 26 sets of *Campylobacter* results



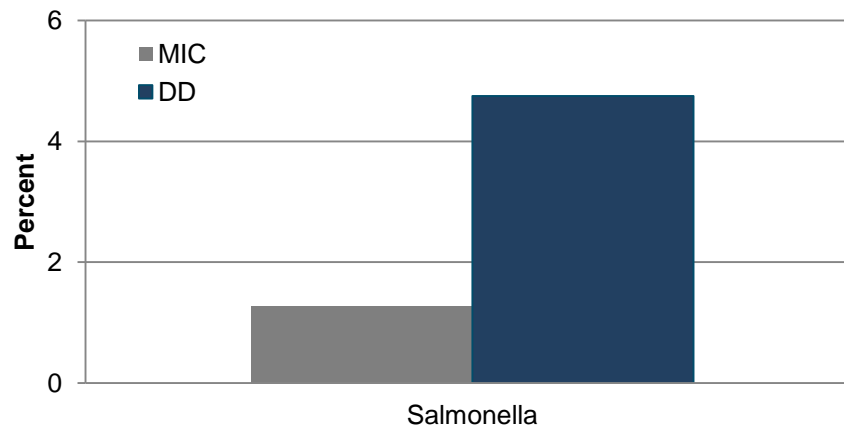
S-6.2/streptomycin and S-6.6/streptomycin

- If only 75% of the results were correct (strain/antimicrobial combination) => further analysis of data:



Methods and guidelines

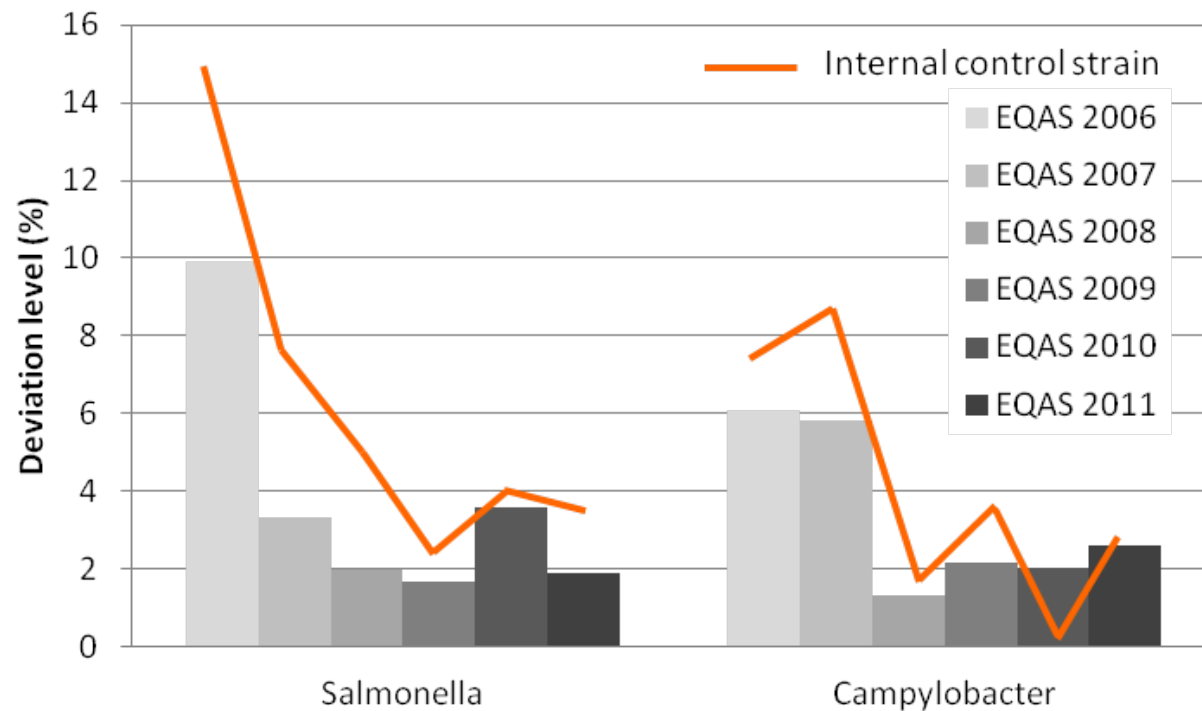
- MIC methods for *Salmonella* AST are recommended
- For *Campylobacter* AST, MIC methods, only, are accepted
- Interpretation guidelines for MIC results are given in the protocol
- For interpretation of zone diameters (*Salmonella*), the laboratory's routine should be followed (S/R)



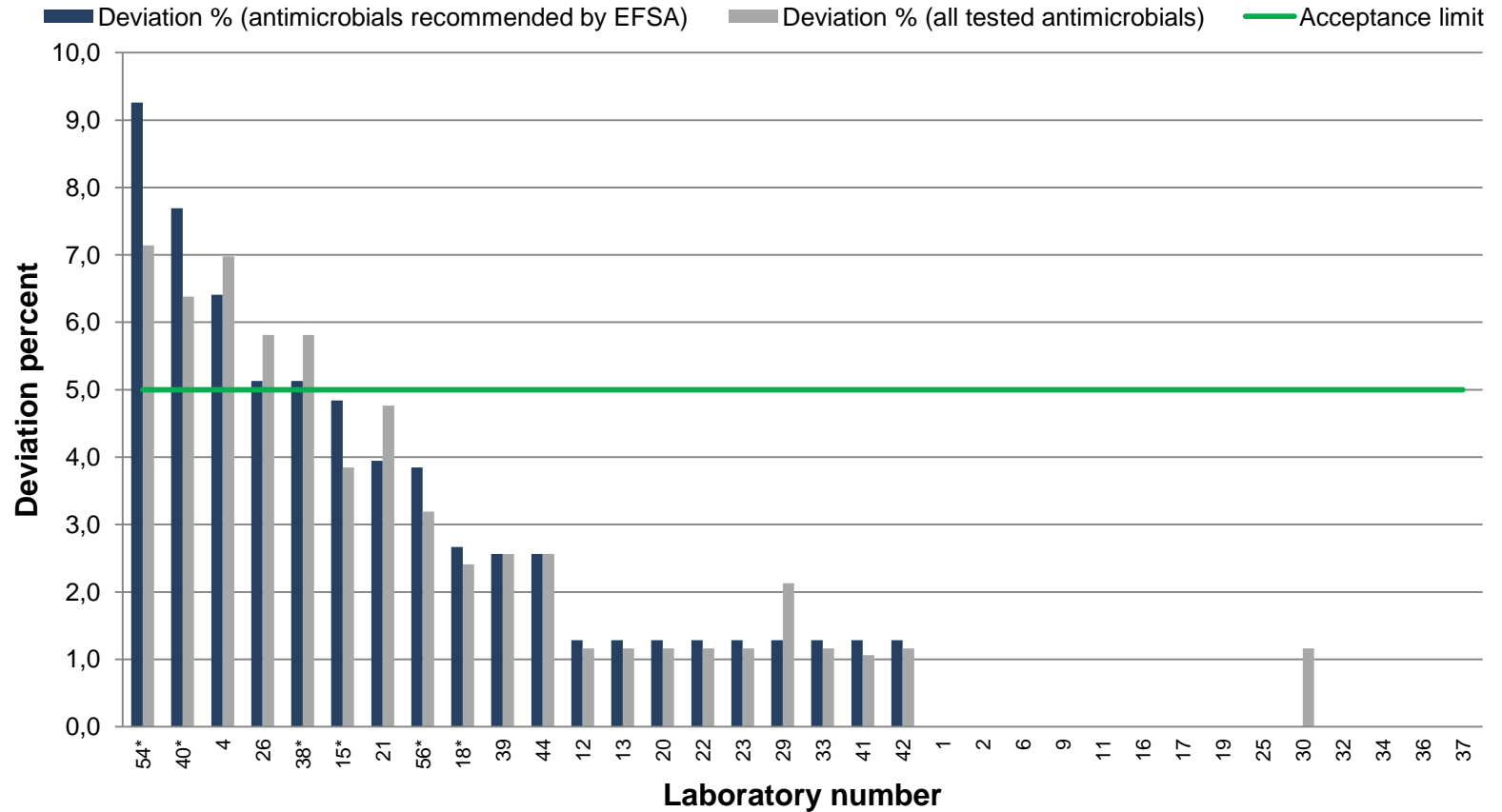
Significant difference between performance; MIC and DD ($p < 0.01$)



Comparison to former EQASs



Salmonella results – pr. lab



Salmonella – deviations on antimicrobials

EQAS 2011	% correct
Ampicillin, AMP	100.0
Cefotaxime, CTX	98.9
Ceftazidime, CAZ*	97.8
Ceftiofur, XNL*	98.4
Chloramphenicol, CHL	100.0
Ciprofloxacin, CIP	94.3
Gentamicin, GEN	98.9
Nalidixic acid, NAL	97.7
Streptomycin, STR	97.0
Sulphonamides, SMX	95.4
Tetracycline, TET	99.3
Trimethoprim, TMP	99.6



*Not part of EFSA monitoring programme



CIP – towards *Salmonella* – cut off

- Ciprofloxacin (5.7% deviation; 15/264)
 - Low cut-off value is used: $R > 0.06 \mu\text{g/mL}$
(lower than the CLSI clinical breakpoint)

=> when performing DD and using the CLSI interpretative criteria, the resistance is not seen!



CIP – towards *Salmonella* – method

- Disk diffusion: 13/15 deviations
- MIC-determination: 2/15 deviations

If performing DD for AST:

- Check the nalidixic acid result
 - ⇒ If Nal-R, ciprofloxacin should also be interpreted resistant (see protocol)
 - ⇒ If Nal-S and reduced susceptibility (<30mm) towards CIP, you may want to check for a plasmid mediated quinolone resistance gene (e.g. by PCR)
(see: **Cavaco** and Aarestrup. J Clin Microbiol. 2009 Sep;47(9):2751-8)



QC strains – *Salmonella*, MIC

EQAS 2011 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	0/28 (0%)	-	-
Cefotaxime, CTX	0/28 (0%)	-	-
Cefoxitin, FOX	0/6 (0%)	-	-
Ceftazidime, CAZ	0/22 (0%)	-	-
Ceftiofur, XNL	0/3 (0%)	-	-
Chloramphenicol, CHL	0/28 (0%)	-	-
Ciprofloxacin, CIP	4/28 (14%)	-	1 step
Gentamicin, GEN	0/28 (0%)	-	-
Imipenem, IMI	0/3 (0%)	-	-
Nalidixic acid, NAL	0/28 (0%)	-	-
Streptomycin, STR	0/27 (0%)	-	-
Sulphonamides, SMX	1/20 (5%)	-	1 step
Tetracycline, TET	0/28 (0%)	-	-

All laboratories performing MIC uploaded results for the QC-strain



QC strains – *Salmonella*, disc diffusion

EQAS 2011 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	0/4 (0%)	-	-
Cefotaxime, CTX	0/5 (0%)	-	-
Cefoxitin, FOX	0/4 (0%)	-	-
Ceftazidime, CAZ	0/5 (0%)	-	-
Ceftiofur, XNL	0/4 (0%)	-	-
Chloramphenicol, CHL	0/5 (0%)	-	-
Ciprofloxacin, CIP	0/4 (0%)	-	-
Gentamicin, GEN	0/5 (0%)	-	-
Imipenem, IMI	0/2 (0%)	-	-
Nalidixic acid, NAL	0/5 (0%)	-	-
Streptomycin, STR	0/5 (0%)	-	-
Sulphonamides, SMX	0/3 (0%)	-	-
Tetracycline, TET	0/5 (0%)	-	-
Trimethoprim, TMP	0/4 (0%)	-	-

One laboratory performing DD did not upload results for the QC-strain



ESBL-producing test strains

ESBL-detection is mandatory in the *Salmonella* (and *E. coli*) EQASs

Percentage reporting correct ESBL-results

	Strain S-6.1 (CTX M-52)	Strain S-6.3 (CTX M-15/SHV-12)	Strain S-6.4 (CTX M-15 like)	Strain S-6.8 (CMY-2)
Confirmed ESBL-producer	32/34 (94%)	32/34 (94%)	32/34 (94%)	2/34 (6%)
FOX^R	-	-	-	31/34 (91%)
ampC confirmed	-	-	-	30/34 (88%)
ampC not confirmed	34/34 (100%)	34/34 (100%)	34/34 (100%)	4/34 (12%)

- One lab did not perform ESBL-detection at all



CMY-2 producing test strain

	Strain S-6.8 (CMY-2)	
Confirmed ESBL-producer	2/34 (6%)	#4 and #22
FOX ^R	29/34 (91%)	
ampC confirmed	30/34 (88%)	
ampC not confirmed	4/34 (12%)	#38, #39, #41, #54

Confirmed as both
ESBL and ampC-
producer

Cephalosporin resistance was
detected – no indication of
reaction to this



ESBL-producing test strains – detection

Proportion of laboratories successfully using different cephalosporins for screening (correct confirmation of ESBL production)

	Strain S-6.1 (CTX M-52)	Strain S-6.3 (CTX M-15/SHV-12)	Strain S-6.4 (CTX M-15 like)	Strain S-6.8 (CMY-2)
CTX, CAZ, XNL	6/7 (86%)	6/7 (86%)	5/7 (71%)	5/7 (71%)
CTX, CAZ	21/22 (95%)	21/22 (95%)	22/22 (100%)	21/22 (95%)
CTX, XNL	1/1 (100%)	1/1 (100%)	1/1 (100%)	1/1 (100%)
CTX	4/4 (100%)	4/4 (100%)	4/4 (100%)	3/4 (75%)

No confirmatory testing

Mix up of strains

No cefoxitin results



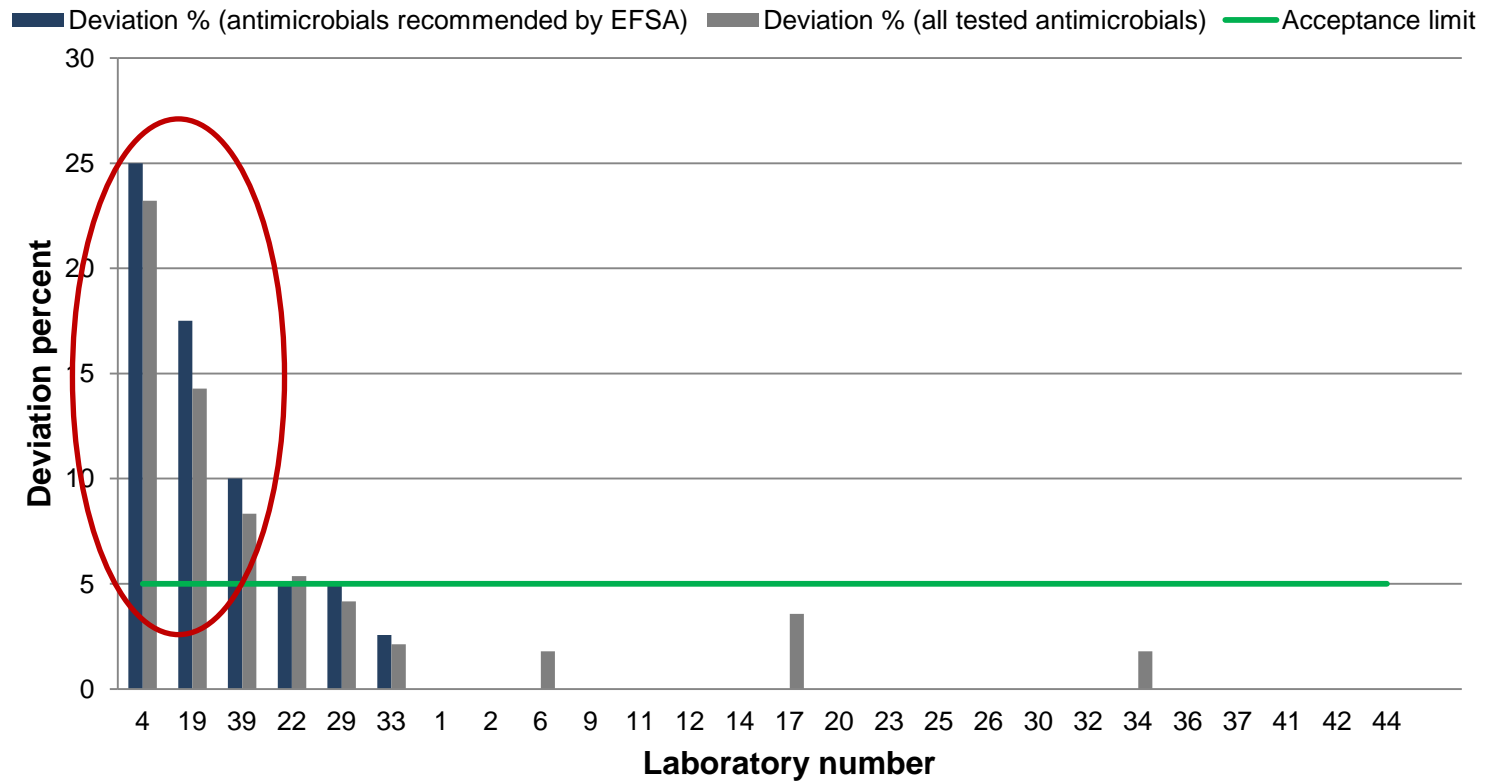
Campylobacter – deviations on antimicrobials

	% correct
Chloramphenicol, CHL*	100.0
Ciprofloxacin, CIP	99.0
Erythromycin, ERY	98.0
Gentamicin, GEN	99.0
Nalidixic acid, NAL*	95.5
Streptomycin, STR	93.1
Tetracycline, TET	98.5

*Not part of EFSA monitoring programme



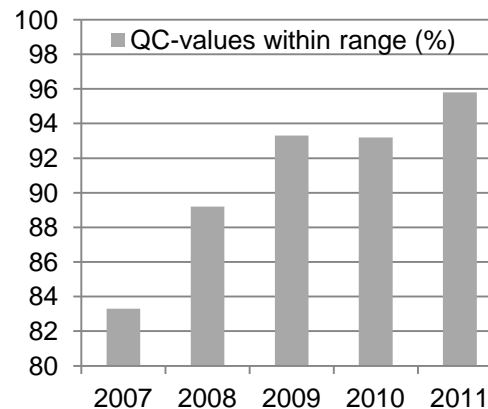
Campylobacter results – pr. lab



QC strains – *Campylobacter*, MIC

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	0/19 (0%)	-	-
Ciprofloxacin, CIP	0/25 (0%)	-	-
Erythromycin, ERY	0/25 (8%)	-	-
Gentamicin, GEN	3/24 (13%)	1 step	3 steps
Nalidixic acid, NAL	1/25 (0%)	2 steps	-
Tetracycline, TET	2/25 (8%)	-	1 step

All 26 labs
uploaded QC-
strain data on
Campy



Genotypic characterisation - background

Strains

Staphylococcus aureus

Salmonella spp.

Method

Participants were encouraged to use their own laboratory's method(s) for the testing.

(Appendix 11: References and primer-sequences)

Expected results (identified genes)

AST-profile + microarray. Selected results confirmed by PCR

Verification of results

None



Genotypic characterisation – GEN-3.1 (G-pos)

- Four laboratories participated
- All participating laboratories obtained satisfying results

	Lab I	Lab III	Lab IV	Lab VII
aadE or aad(6)*	NT	NT	NT	NT
blaI	NT	1	1	NT
blaR	NT	1	1	NT
blaZ	1	1	1	NT
mecA	1	1	1	1
vga(A)	1	1	NT	NT
tet(K)	1	1	NT	NT
tet(M)	1	1	1	NT

*aadE and aad(6) are synonyms for the same aminoglycoside resistance gene



Genotypic characterisation – GEN-3.2 (G-neg)

- Six laboratories participated
- All participating laboratories obtained satisfying results

	Lab I	Lab III	Lab IV	Lab VI	Lab VII	Lab VIII
CMY-2	1/1	1/1	1/1	NT	NT	1/1
floR	1	1	1	NT	NT	1
strA	NT	1	1	NT	NT	1
strB	1	1	1	NT	NT	1
aadA	1	1	1	NT	NT	1
sul1	1	1	1	NT	NT	1
sul2	1	1	1	NT	NT	1
tetA	1	1	1	NT	NT	1
Additional info				qnr-genes tested, not found	AmpC Cit	



Summing up I

The *Salmonella* trial

- 34 labs participated
- 29 labs performed with deviation levels < 5%
- No outliers

Challenges:

Ciprofloxacin (disk diffusion)

Detection and confirmation of ESBL-producers



Summing up II

The *Campylobacter* trial

- 26 labs participated
- 23 labs performed with deviation levels $< 5\%$
- Three defines as outliers



Summing up III

The genotypic characterisation

- Good agreement with the expected
- Few participants

Thanks for your attention!

Thoughts? Questions?

