



**DTU Food**  
National Food Institute

# EQAS 2009

*Salmonella and Campylobacter*

EURL workshop, April 8-9<sup>th</sup>, 2010

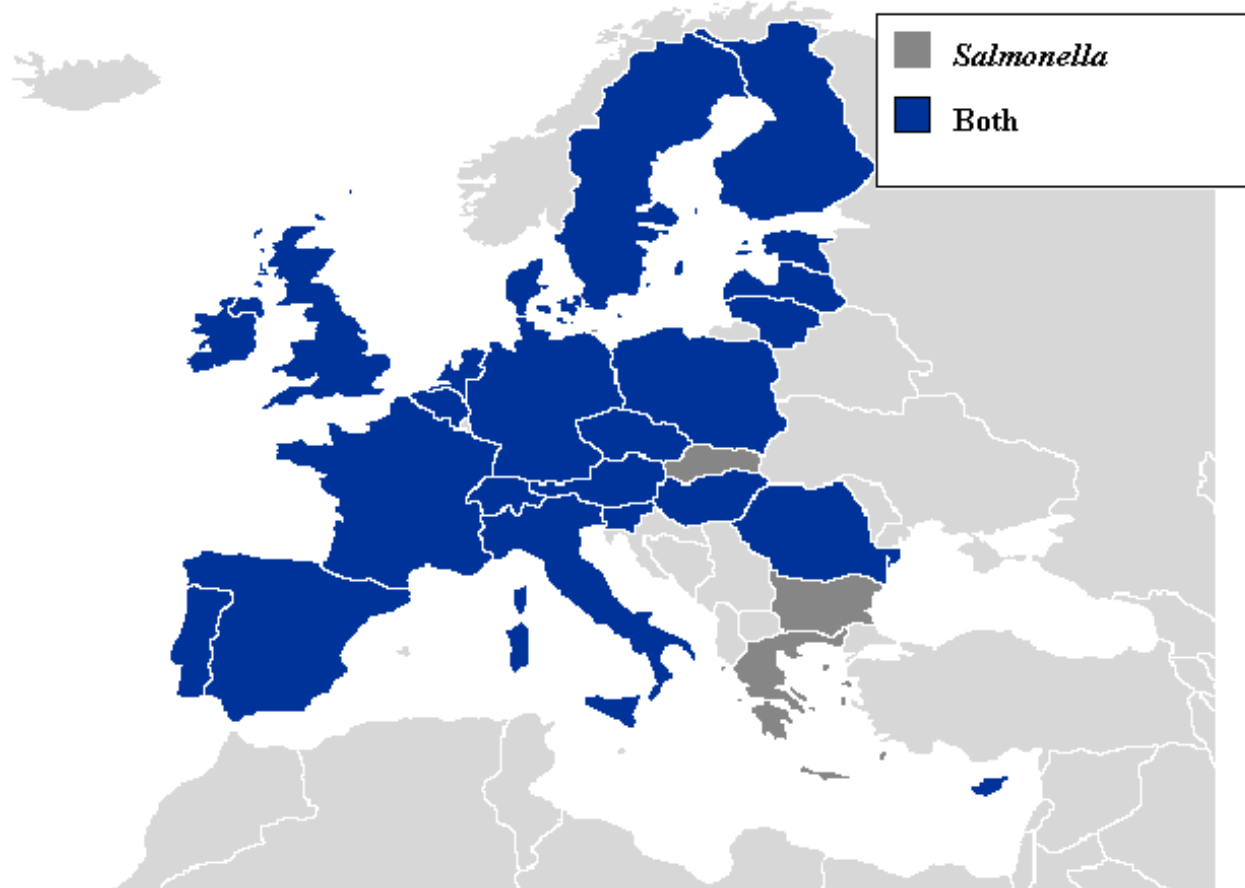
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## Outline of Salm/Camp EQAS 2009

- AST of eight *Salmonella* and eight *Campylobacter*
- New participants were supplied with original reference strains ATCC 25922 and ATCC 33560 for QC testing
- Instant individual evaluation report
- Report comparing and evaluating all results
- (If only 75% of the results were correct (strain/antimicrobial combination) => further analysis of data)
- Aim: That all NRL's perform AST with less than 5% incorrect interpretations
- Optional: genotypic characterization of a multidrug resistant *Salmonella* strain

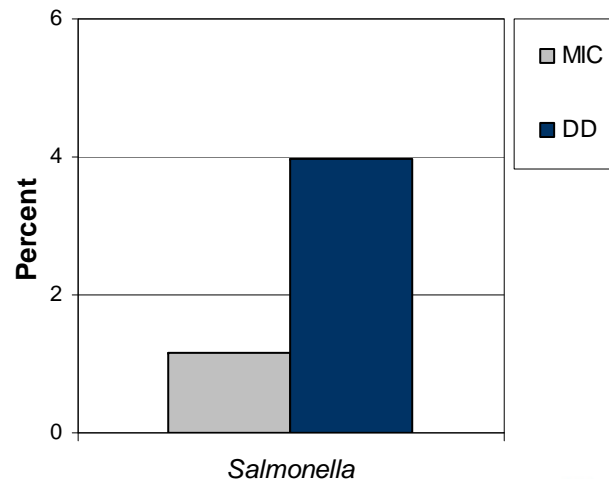


## Participation in the Salm/Camp EQAS, 2009

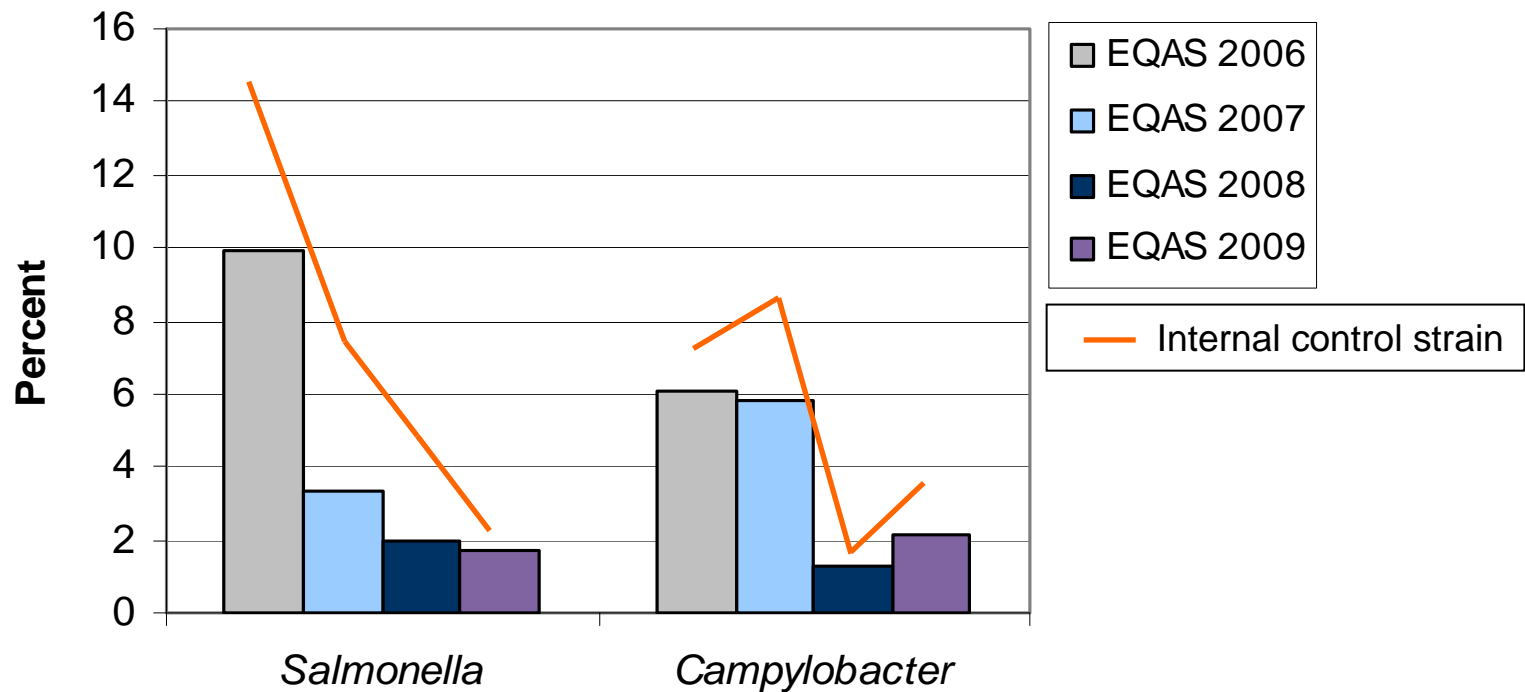


## Methods and guidelines

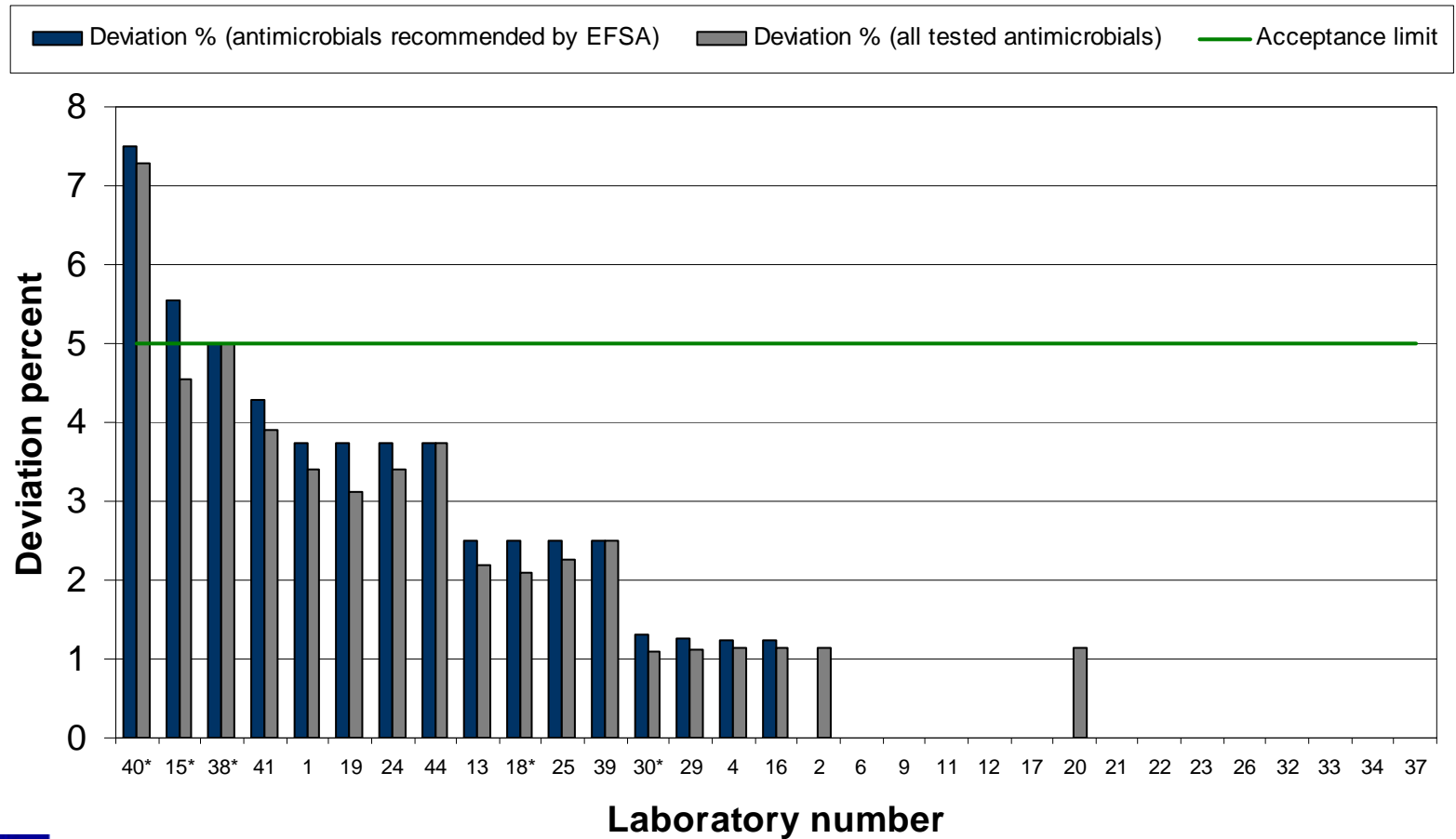
- MIC methods for *Salmonella* AST are recommended
- For *Campylobacter* AST, MIC methods, only, are accepted
- Interpretation guidelines for MIC results are given in the protocol
- For interpretation of zone diameters (*Salmonella*), the laboratory's routine should be followed (however 'susceptible'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



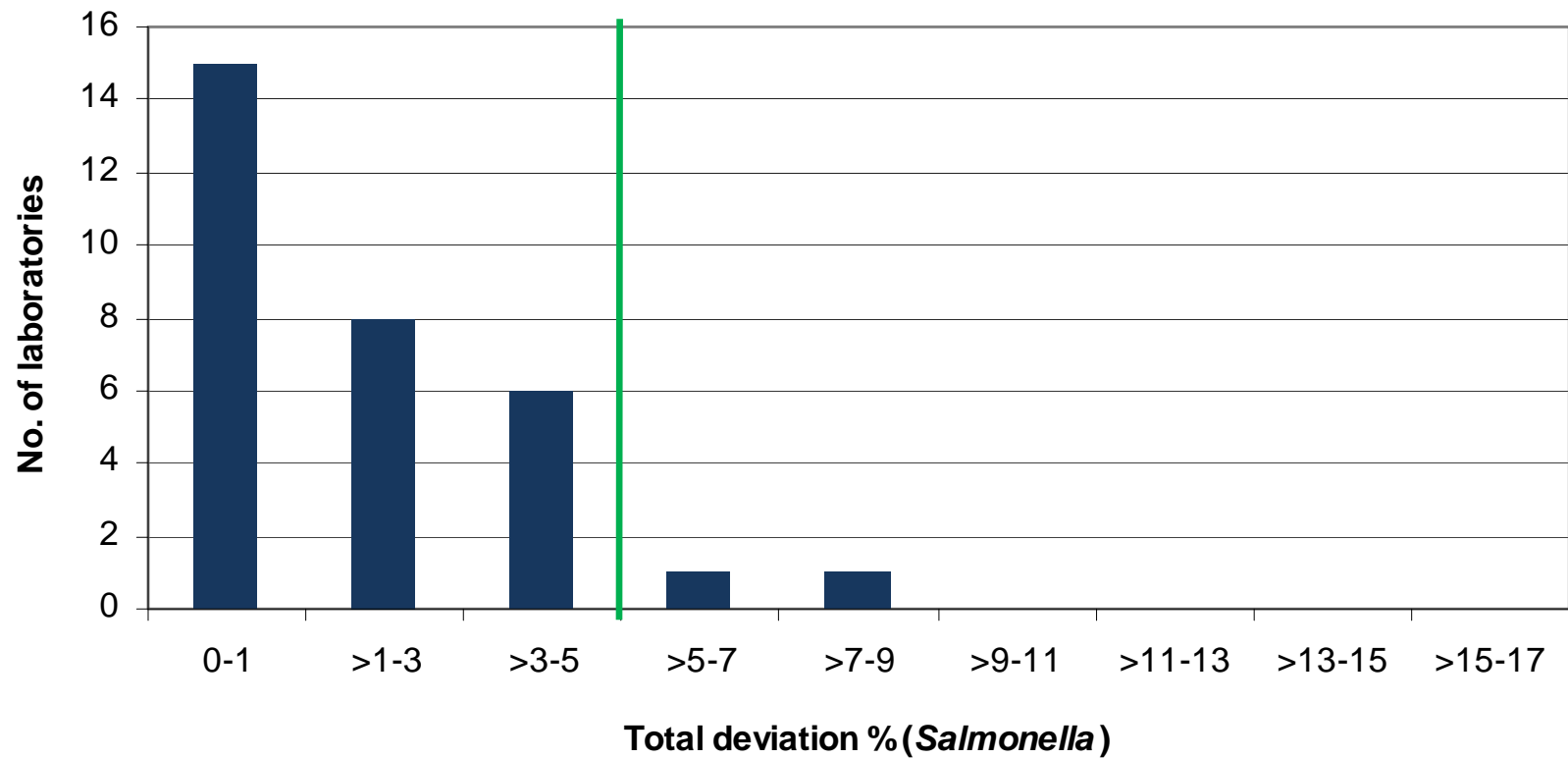
## Comparison to former EQASs



## Salmonella results – pr. lab



## Salmonella results – intervals



## *Salmonella* – deviations on antimicrobials

	% correct
Ampicillin, AMP	99.6
Cefotaxime, CTX	99.2
Ceftazidime, CAZ	98.3
Ceftiofur, XNL	100.0
Chloramphenicol, CHL	99.2
Ciprofloxacin, CIP	94.5
Gentamicin, GEN	99.6
Nalidixic acid, NAL	99.6
Streptomycin, STR	95.3
Sulphonamides, SMX	98.7
Tetracycline, TET	97.1
Trimethoprim, TMP	100.0

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





## CIP – towards *Salmonella*

- Ciprofloxacin (5.5% deviation; **13/238**)
  - 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!
  - Disk diffusion: 6/13 deviations (3 on S-4.6)
  - MIC-determination: 7/13 deviations (4 on S-4.6)  
Four out of seven MIC's were actually  $>$  the cut off!
- 7/13 deviations were on S-4.6 – harbouring *qnrB*  
(Nal-S and CIP-low-level-R)



## CIP – if performing DD

- 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 also:

**Cavaco LM**, Aarestrup FM. Evaluation of quinolones for use in detection of determinants of acquired quinolone resistance, including the new transmissible resistance mechanisms *qnrA*, *qnrB*, *qnrS*, and *aac(6')Ib-cr*, in *Escherichia coli* and *Salmonella enterica* and determinations of wild-type distributions. **J Clin Microbiol.** 2009 Sep;47(9):2751-8

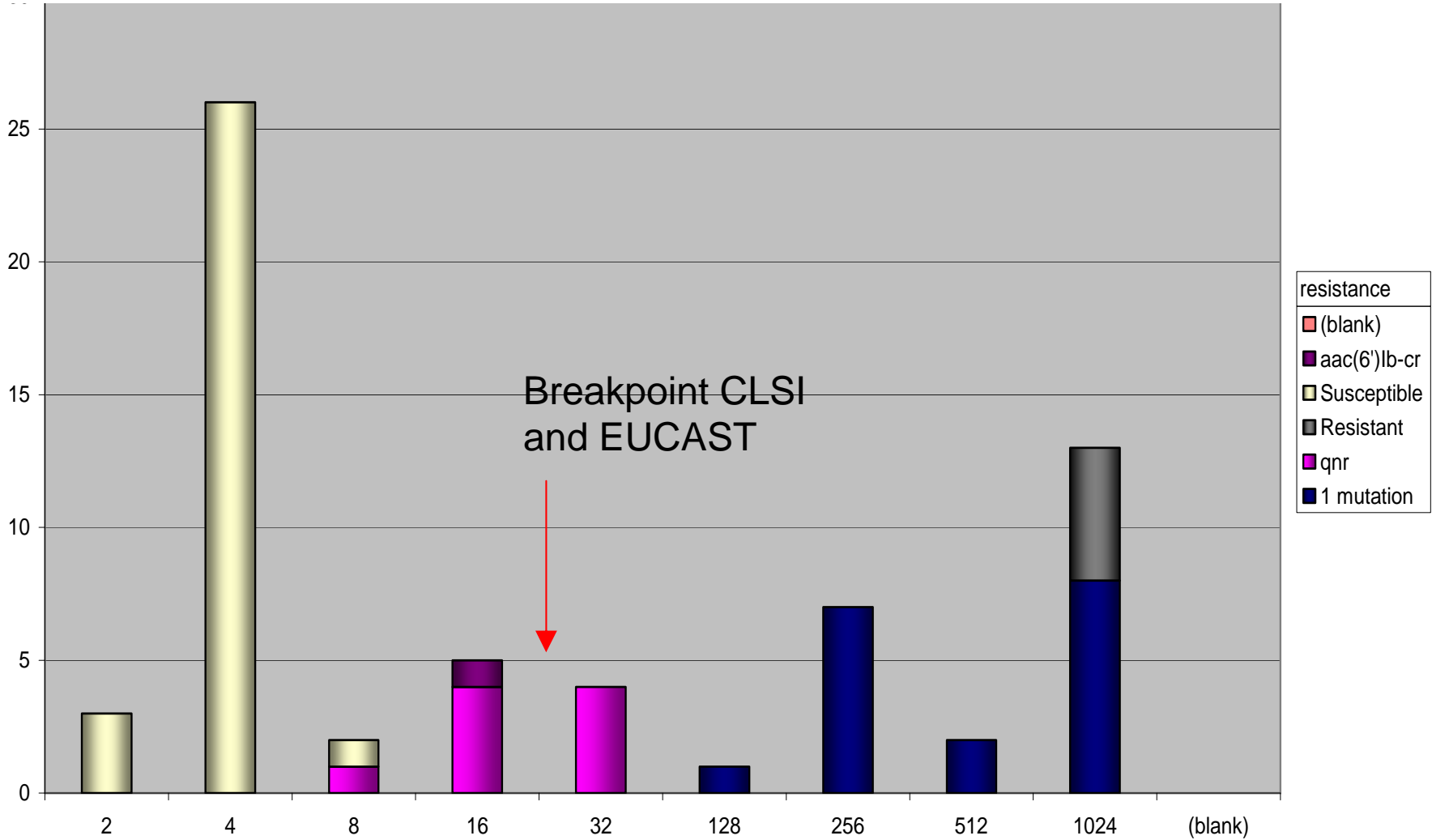


## Quinolone low level resistance

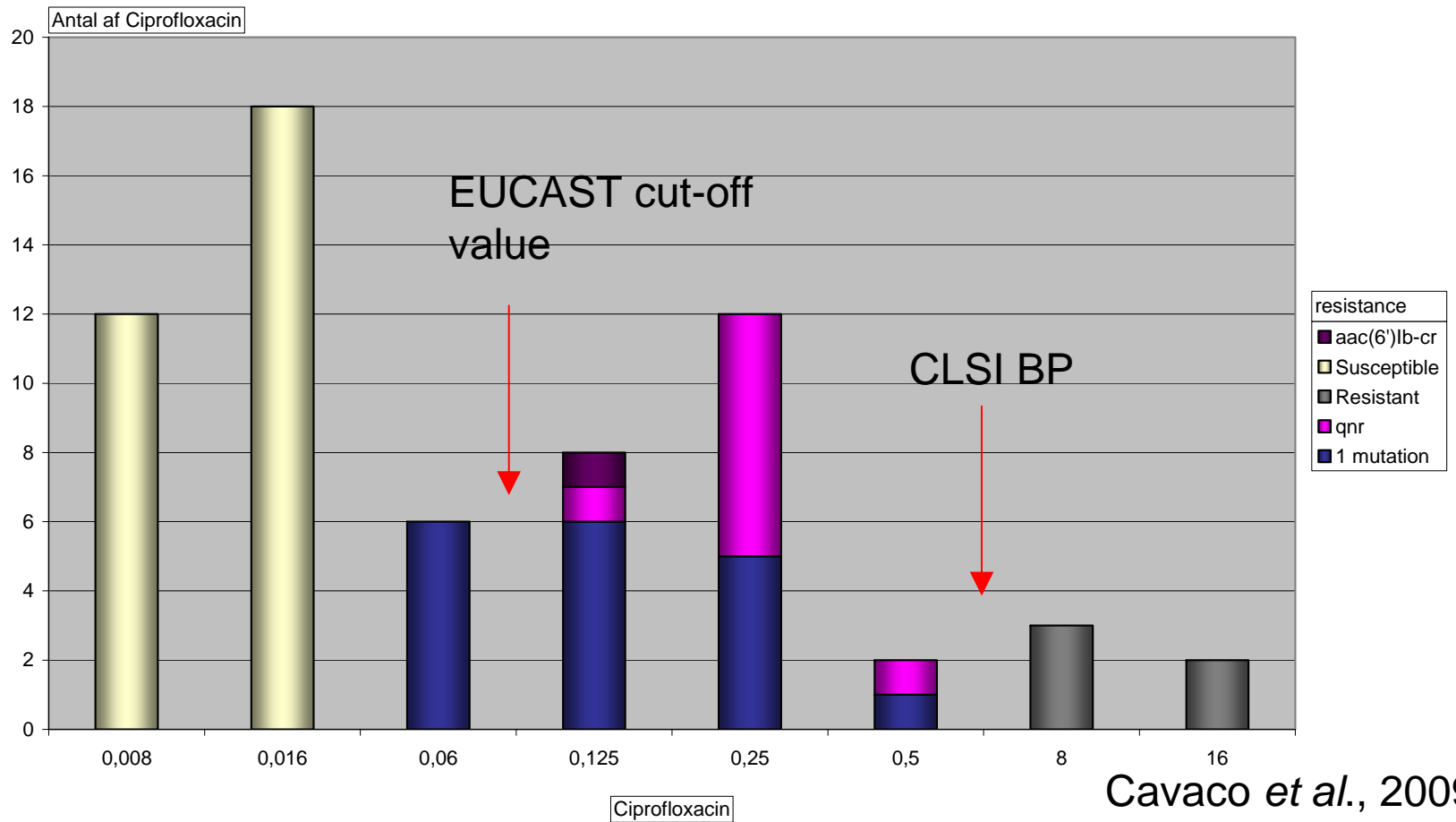
- **Target modification** – Nal-R good indicator, stepwise increase in fluoroquinolone resistance (depends on no. of mutations)
- **Target protection (*qnr*)** – low level resistance towards fluoroquinolones but nalidixic acid not so much affected
- **Enzymatic modification (*aac(6')Ib-cr*)** – low level resistance towards ciprofloxacin and norfloxacin MIC of other quinolones not affected
- **Specific efflux (*qepA*)** – Low-level reduction in susceptibility of some fluoroquinolones (hydrophilic)



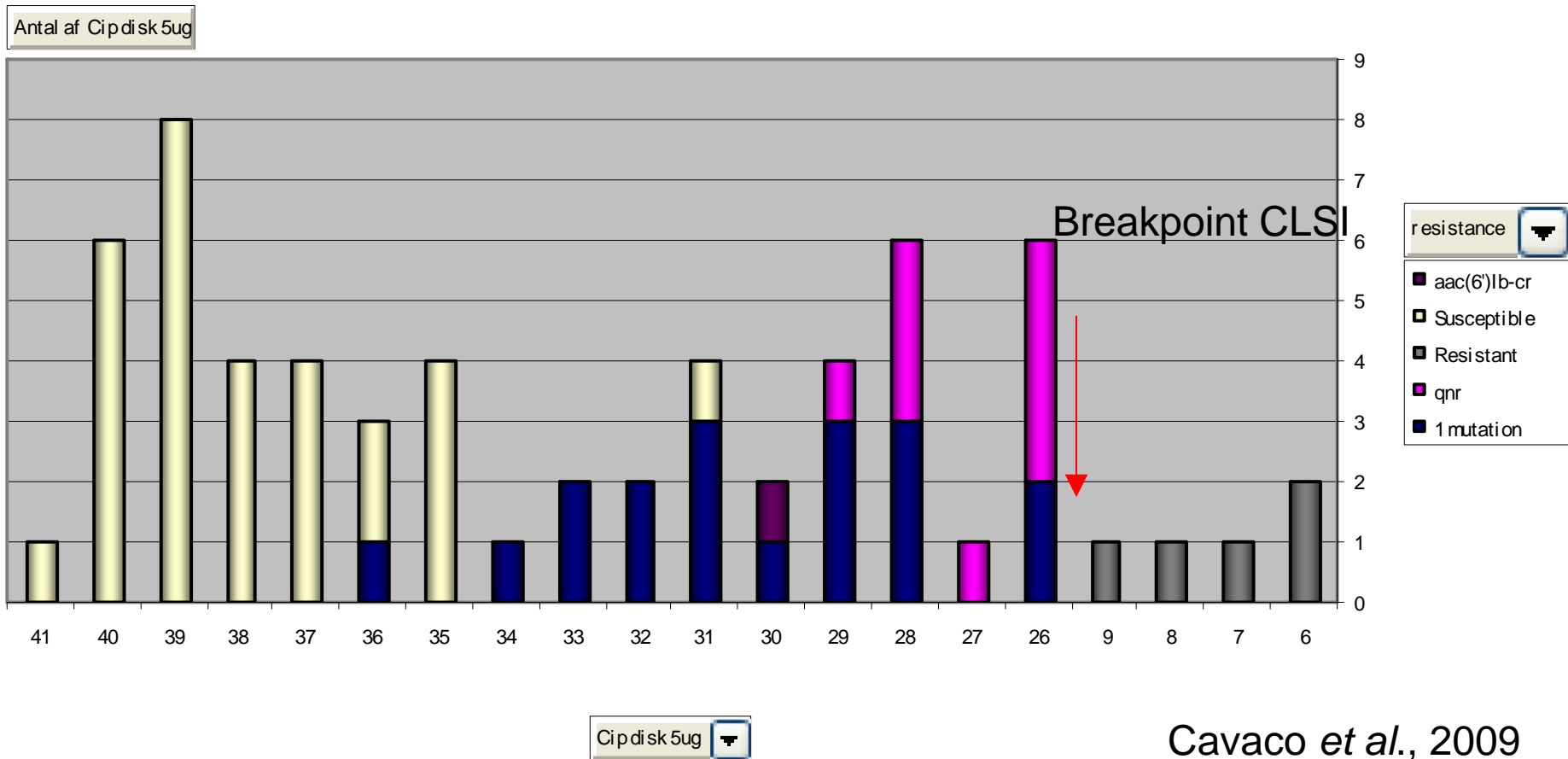
# MIC – Nalidixic acid



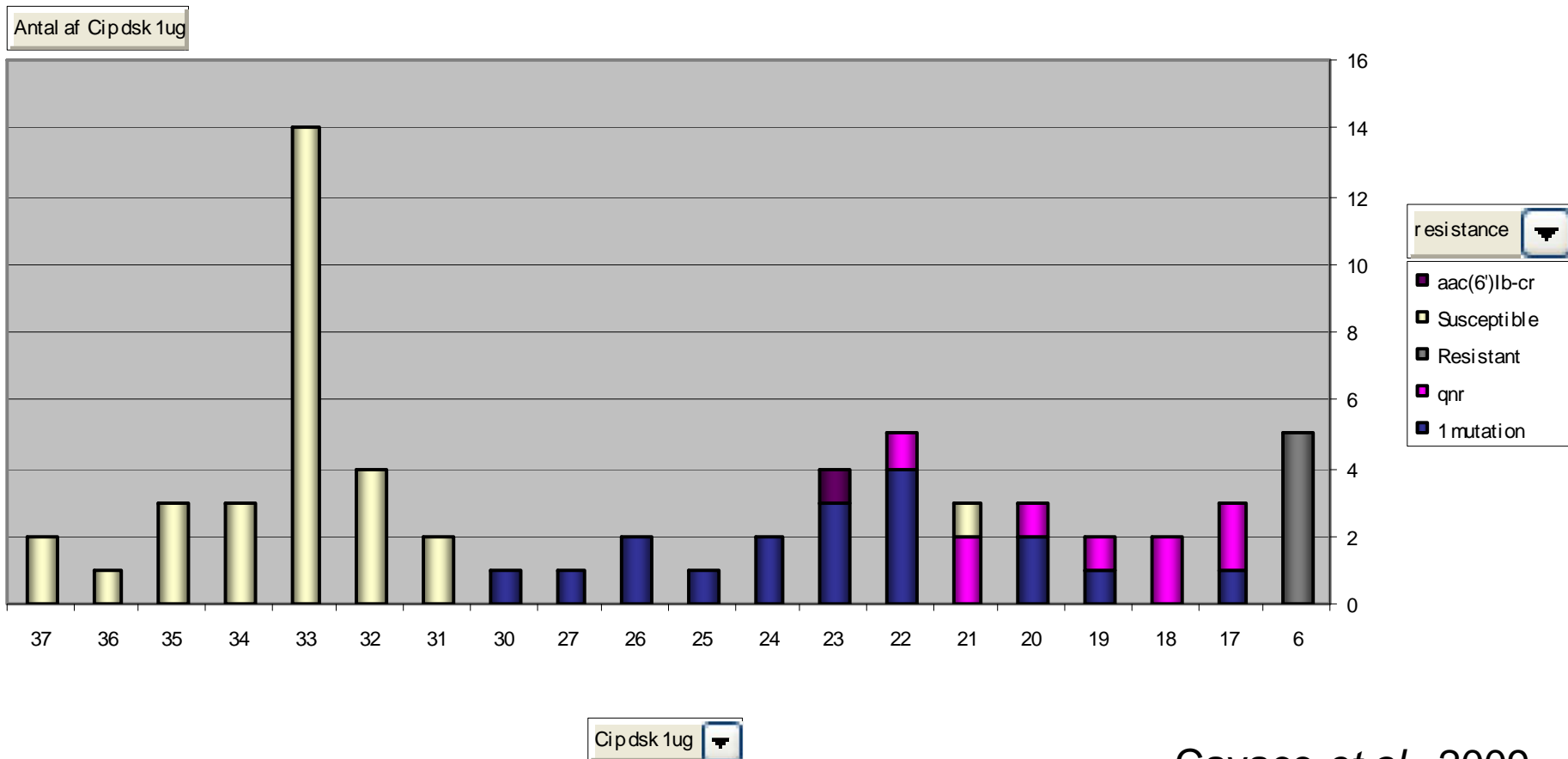
# MIC- Ciprofloxacin



# Disk diffusion- Ciprofloxacin 5 µg



# Disk diffusion- Ciprofloxacin 1 $\mu$ g



## Plasmid mediated Quinolone Resistance phenotype

### Fluoroquinolone low-level resistance

- MIC of ciprofloxacin: 0.125-1  $\mu\text{g}/\text{mL}$
- DD inhibition zone of ciprofloxacin:
  - 5 $\mu\text{g}$ : 26-30mm or
  - 1 $\mu\text{g}$ : 17-23 mm

### Nalidixic acid susceptible or intermediate

- MIC of nalidixic acid: 4-32  $\mu\text{g}/\text{mL}$
- DD inhibition zone of nalidixic acid:  
13-19mm (mutants have usually no inhibition zone)





## QC strains – *Salmonella*, MIC

EQAS 2009  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	1/26 (4%)	-	1 step
Cefotaxime, CTX	1/26 (4%)	-	2 steps
Cefoxitin, FOX	2/4 (50%)	2 steps	1 step
Ceftazidime, CAZ	1/19 (5%)	-	3 steps
Ceftiofur, XNL	0/5 (0%)	-	-
Chloramphenicol, CHL	0/26 (0%)	-	-
Ciprofloxacin, CIP	3/26 (12%)	-	1 step
Gentamicin, GEN	1/26 (4%)	-	1 step
Nalidixic acid, NAL	0/26 (0%)	-	-
Streptomycin, STR	0/25 (0%)	-	-
Sulphonamides, SMX	1/13 (8%)	3 steps	-
Tetracycline, TET	1/26 (4%)	-	1 step
Trimethoprim, TMP	0/25 (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	0/5	-	-
Cefotaxime, CTX	1/5	-	1
Cefoxitin, FOX	0/5	-	-
Ceftazidime, CAZ	0/3	-	-
Ceftiofur, XNL	1/3	1	-
Chloramphenicol, CHL	0/5	-	-
Ciprofloxacin, CIP	0/4	-	-
Gentamicin, GEN	1/5	-	1
Imipenem, IMI	1/4	-	8
Nalidixic acid, NAL	0/5	-	-
Streptomycin, STR	0/5	-	-
Sulphonamides, SMX	0/3	-	-
Tetracycline, TET	0/5	-	-
Trimethoprim, TMP	0/5	-	-

## ESBL-producing test strains

- Workshop '08: ESBL-detection should be mandatory in the *Salmonella* and *E. coli* EQASs

Percentage that reported the test strain ESBL-positive

	Strain S-4.1 (CTX M-15)	Strain S-4.2 (CTX M-15)	Strain S-4.5 (CTX M-15 like)
Confirmed ESBL	28/31 (90%)	28/31 (90%)	25/31 (81%)
FOX <sup>s</sup>	31/31 (100%)	31/31 (100%)	31/31 (100%)
AmpC not confirmed	31/31 (100%)	31/31 (100%)	31/31 (100%)

- Three labs did not perform ESBL-detection
- All strains were susceptible to FOX => no deviations regarding AmpC

## ESBL-producing test strains – detection

	Strain S-4.1 (CTX M-15)	Strain S-4.2 (CTX M-15)	Strain S-4.5 (CTX M-15 like)
CTX, CAZ, XNL	5/5 (100%)	5/5 (100%)	5/5 (100%)
CTX, CAZ	17/18 (94%)	17/18 (94%)	15/18 (83%)
CTX, XNL	3/3 (100%)	3/3 (100%)	3/3 (100%)
CTX	3/5 (60%)	3/5 (60%)	2/5 (40%)

- Strain S-4.5: One lab found CTX susceptible  
Two labs found CTX resistant, CAZ susceptible

=> Reminder: when detecting ESBL-producing strains in the EQAS: If a microorganism is resistant to one or two of CTX, CAZ and/or XNL, it should be regarded resistant to all three (does not include FOX)

## Confirmation of ESBL-production – methods

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

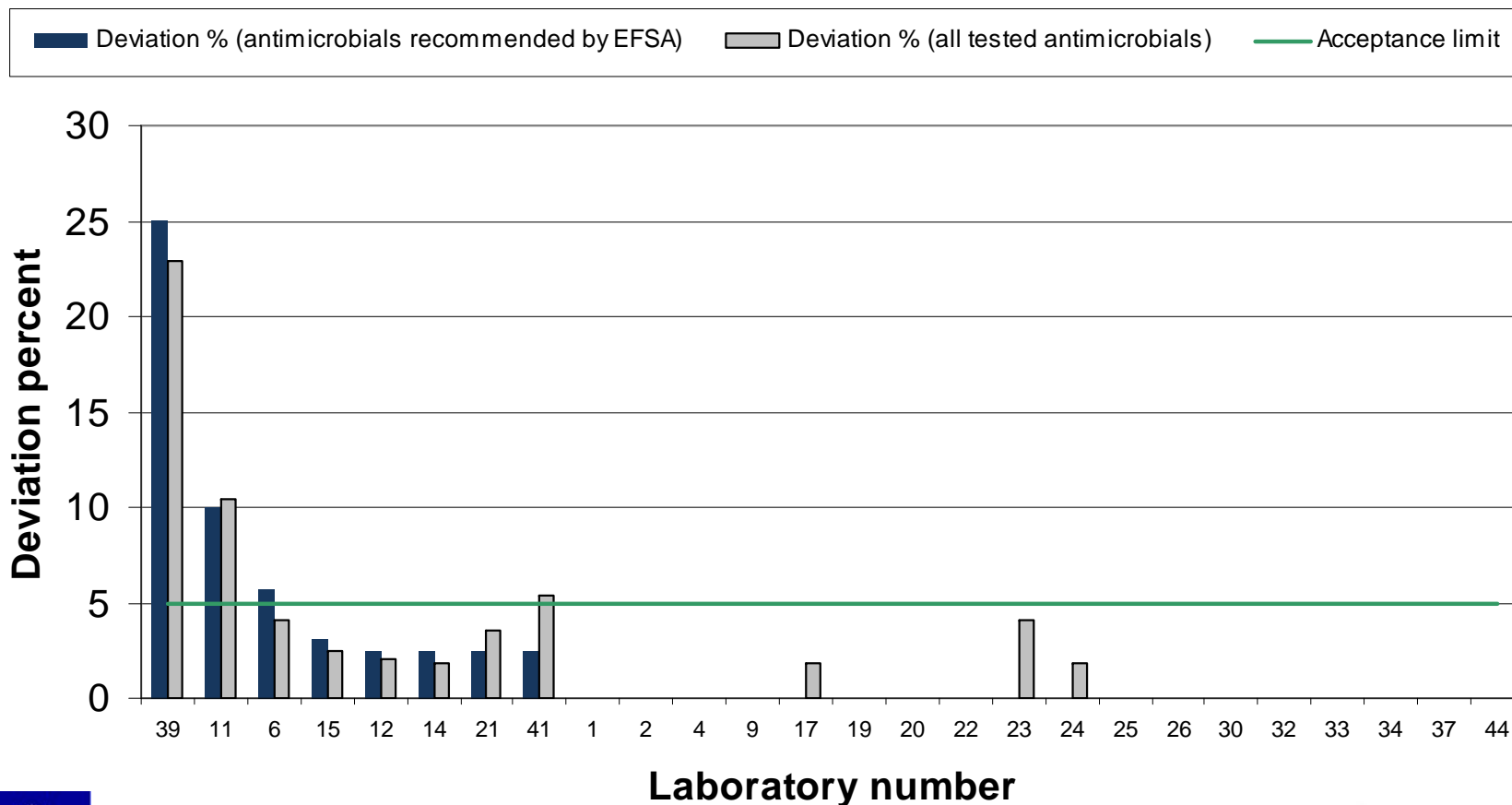
		Increase in zone diameter	MIC-ratio
Expected result /	CAZ:CAZ/CI	41/41 (100%)	33/33 (100%)
no. of results in total	CTX:CTX/CI	44/45 (98%)	37/37 (100%)

Strain	Antimicrobial	Obtained	Expected
CRL S-4.5	CTX:CTX/CI incr. in zone dia.	< 5 mm	>= 5 mm
CRL S-4.5	CAZ:CAZ/CI incr. in zone dia.	< 5 mm	< 5 mm

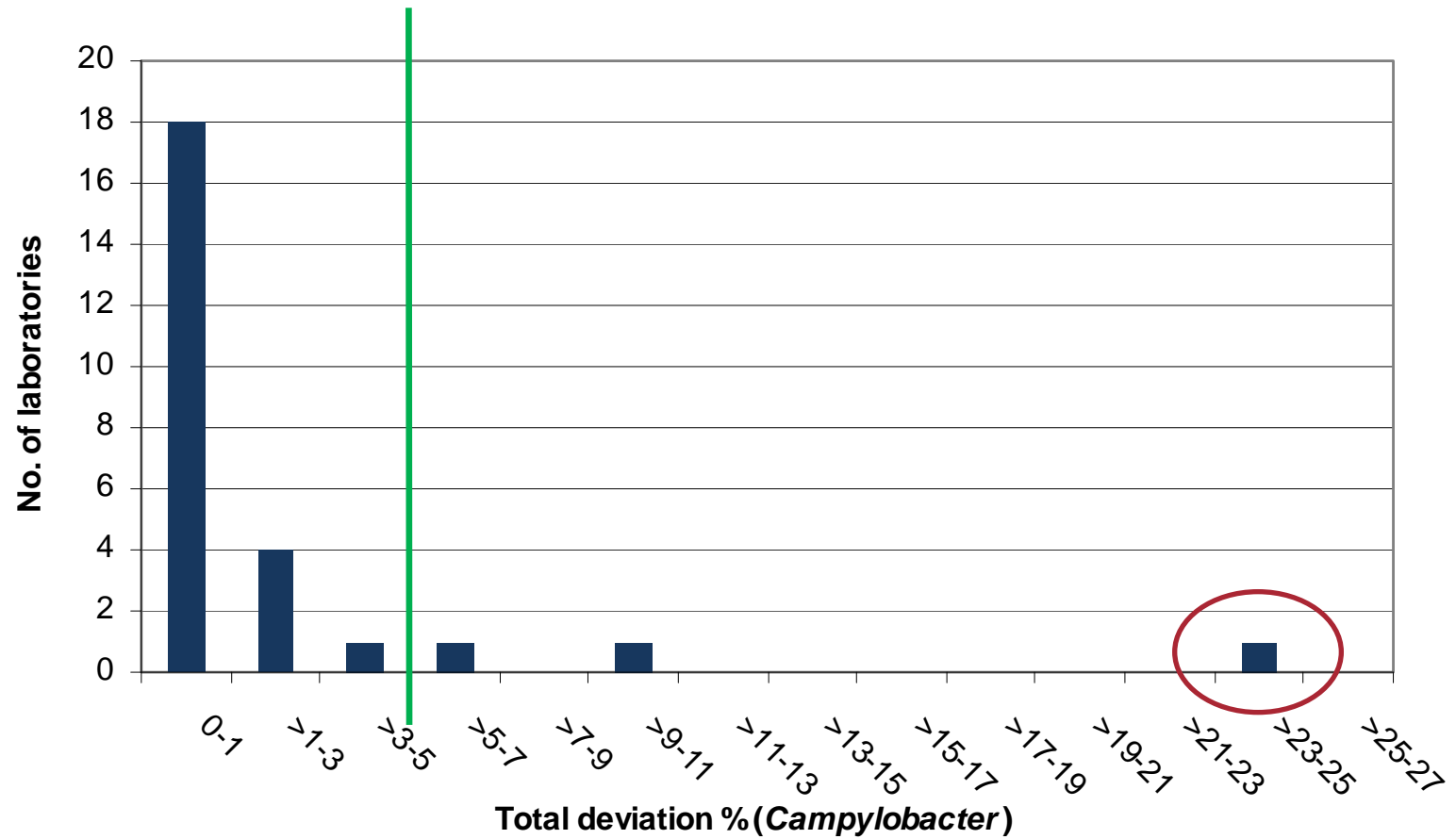
## *Campylobacter* – deviations on antimicrobials

EQAS 2009	% correct
Chloramphenicol, CHL	99.4
Ciprofloxacin, CIP	98.5
Erythromycin, ERY	97.0
Gentamicin, GEN	98.5
Nalidixic acid, NAL	96.5
Streptomycin, STR	98.9
Tetracycline, TET	96.5

## *Campylobacter* results – pr. lab



## *Campylobacter* results – intervals





## QC strains – *Campylobacter*, MIC

EQAS 2009  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	0/18 (0%)	-	-
Ciprofloxacin, CIP	2/24 (8%)	-	2 steps
Erythromycin, ERY	1/24 (4%)	1 step	-
Gentamicin, GEN	3/24 (13%)	2 steps	-
Nalidixic acid, NAL	2/22 (9%)	1 step	2 steps
Tetracycline, TET	1/22 (5%)	-	1 step

- 24 out of 26 labs uploaded QC-strain data on Campy
- Proportion within the QC intervals:
 

2007:	83.8%
2008:	89.2%
2009:	93.3%

## Genotypic characterisation - background

**Strain:** *Salmonella* Concord exhibiting resistance to ampicillin, cefalotin, cefpodoxime, ceftiofur, ceftriaxone, chloramphenicol, ciprofloxacin, gentamicin, streptomycin, sulfamethoxazole, tetracycline, and trimethoprim

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

**Expected results (identified genes):** Obtained by microarrays. Weak results were confirmed by PCR. The results had not been verified elsewhere



## Genotypic characterisation - results

- Five laboratories participated
- All participating laboratories obtained satisfying results

Expected genes	Lab I	Lab II	Lab III	Lab IV	Lab V
CTX M-15	1/-	1/-	1/1	1/1	1/-
SHV-12	1/-	1/-	1/1	1/1	1/-
TEM-1b	1/-	1/-	1/1	1/1	1/-
floR	1	-	1	1	1
qnrB	1	-	1	1	-
strA	1	-	1	1	1
strB	1	-	1	1	1
sul1	1	-	1	1	1
sul2	1	-	1	1	1
tetA	1	-	1	1	1
tetD	1	-	1	1	1
Additional genes detected	dfrA1	-	aac(3)-Ie	-	-



# Summing up I

## The *Salmonella* trial

- 31 labs participated
- 29 labs performed with deviation levels < the acceptance level
- 15 labs had no deviations (EFSA antimicrobials)
- 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 < the acceptance level
- 18 labs had no deviations (EFSA antimicrobials)
- One laboratory was an outlier (25% deviation)
  - Recently introduced MIC-methods for AST of *Campylobacter*
  - Validation is ongoing



## Summing up III

### The genotypic characterisation

- Increased focus on molecular typing
- EURL suggest to repeat this optional part

# Thanks for your attention!

Thoughts? Questions?

