European Union Reference Laboratory Antimicrobial Resistance

DTU Food
National Food Institute

EQAS 2010

Salmonella, *Campylobacter*
and optional genotypic characterisation

EURL workshop, April 4-5th, 2011

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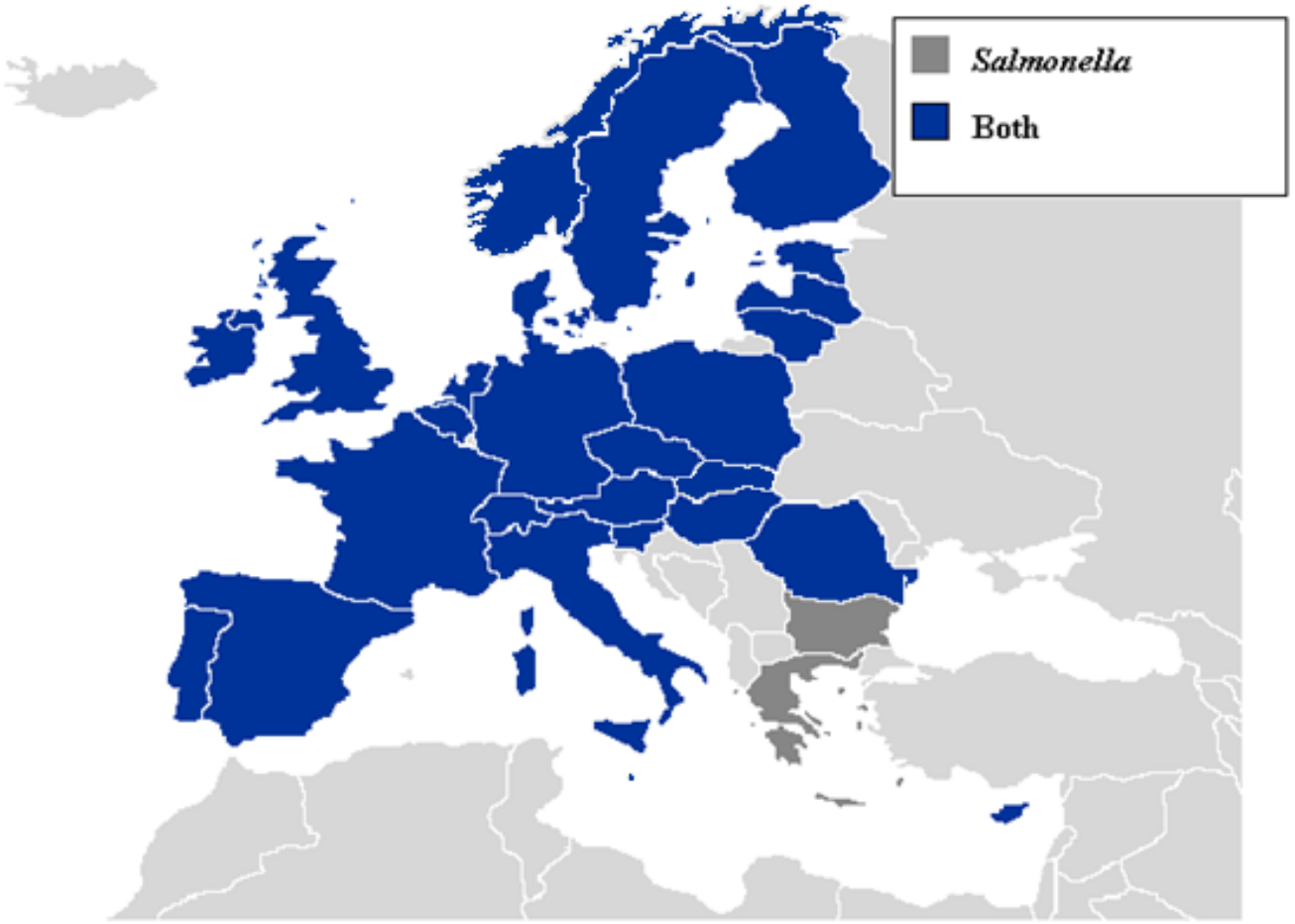
Outline of Salm/Camp EQAS 2010

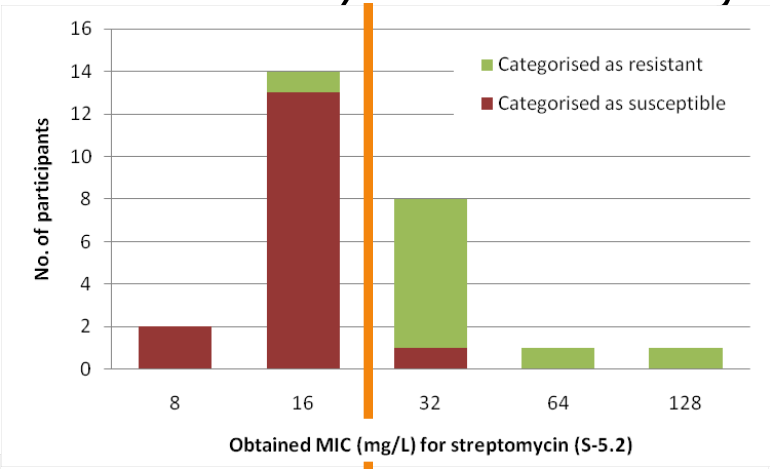
- AST of eight *Salmonella* and eight *Campylobacter*
- New participants were supplied with original reference strains ATCC 25922 and ATCC 33560 for QC testing
- Instant individual evaluation report
- Report comparing and evaluating all results
- Aim: That all NRL's perform AST with less than 5% incorrect interpretations
- Optional: genotypic characterization of a multidrug resistant *Salmonella* strain

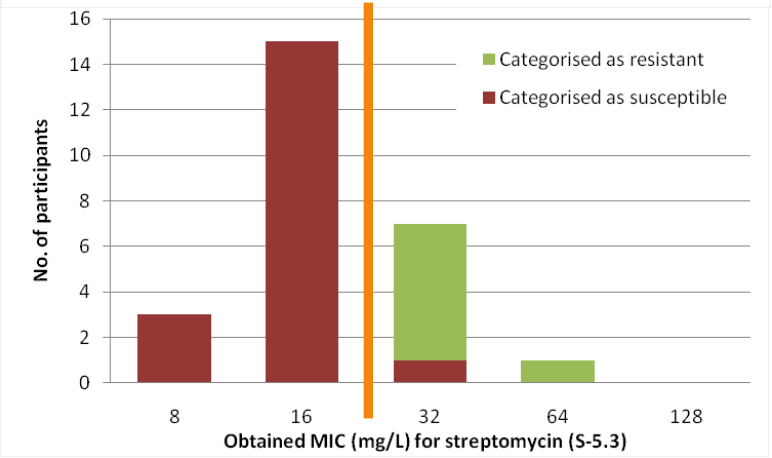
Participation in the Salm/Camp EQAS, 2010

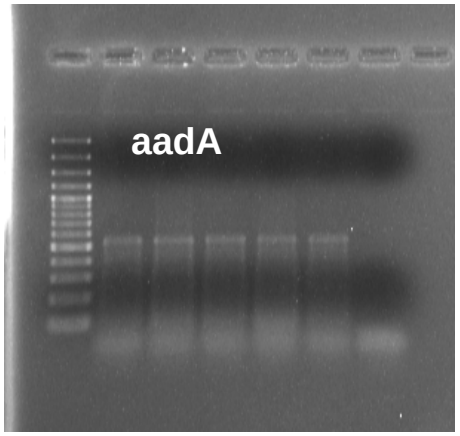


S-5.2/streptomycin and S-5.3/streptomycin

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







M:	100 bp ladder
1	S-5.2 EQAS agarstick
2	S-5.3 EQAS agarstick
3	S-5.2 EQAS from freezer
4	S-5.3 EQAS from freezer
5	Positive control
6	Negative control

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Cut-off values recommended by the
EU Reference Laboratory for Antimicrobial Resistance (EURL-AR)

Standardised cut off values are essential for comparison of antimicrobial susceptibility monitoring results. The European Committee on Antimicrobial Susceptibility Testing (EUCAST; http://www.eucast.org/) provides clinical breakpoints, epidemiological cut-off values and expert rules to assist microbiologists in the interpretation of antimicrobial susceptibility test (AST) results.

For the purpose of monitoring, the EURL-AR recommends the use of EUCAST epidemiological cut-off values, when available, which allow categorisation of bacteria as wildtype or non-wildtype (to simplify, the terms 'susceptible' and 'resistant' are often maintained). Accordingly, the epidemiological cut off values recommended by the EURL-AR for interpretation of AST results for *Salmonella* spp., *Campylobacter coli*, *C. jejuni*, *Escherichia coli*, *Staphylococcus aureus*, *Enterococcus faecium* and *E. faecalis* are listed in Tables 1-5 below.

ESBL producers

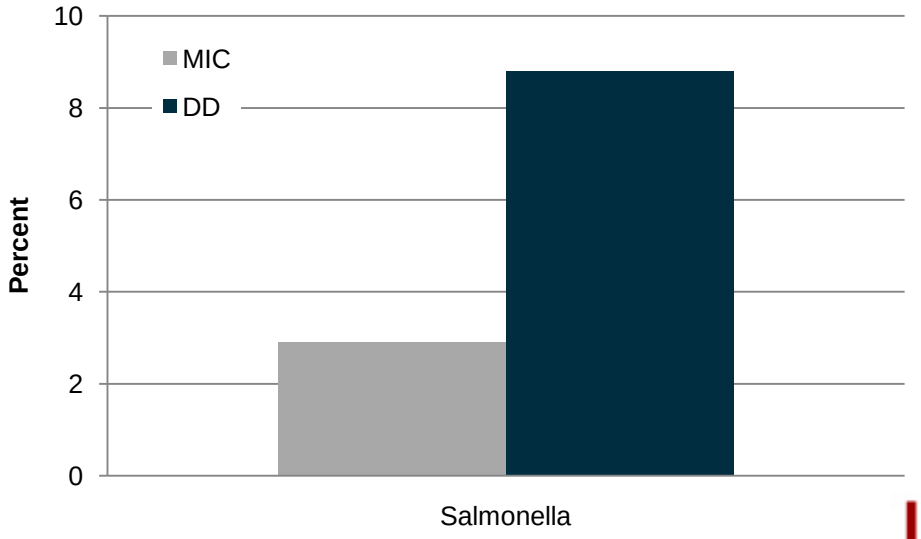
Bacterial isolates resistant to cephalosporins, such as cefotaxime (CTX), ceftazidime (CAZ) or ceftiofur (XNL), should be confirmed as extended-spectrum β-lactamase (ESBL)-producers by confirmatory tests. The EUCAST expert rules state that *the presence or absence of an ESBL does not in itself influence the categorization of susceptibility.*

Table 1: Guidelines for interpretation of antimicrobial susceptibility test results for *Salmonella* spp.

Antimicrobial	MIC (µg/mL) (R>)
Ampicillin (AMP)	8
Cefotaxime (CTX)	0.5
Ceftazidime (CAZ)	2
Ceftiofur (XNL)	2
Chloramphenicol (CHL)	16
Ciprofloxacin (CIP)	0.06
Colistin (COL)	2*
Gentamicin (GEN)	2
Nalidixic acid (NAL)	16
Streptomycin (STR)	16
Sulfonamides (SMX)	256**
Tetracycline (TET)	8

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)



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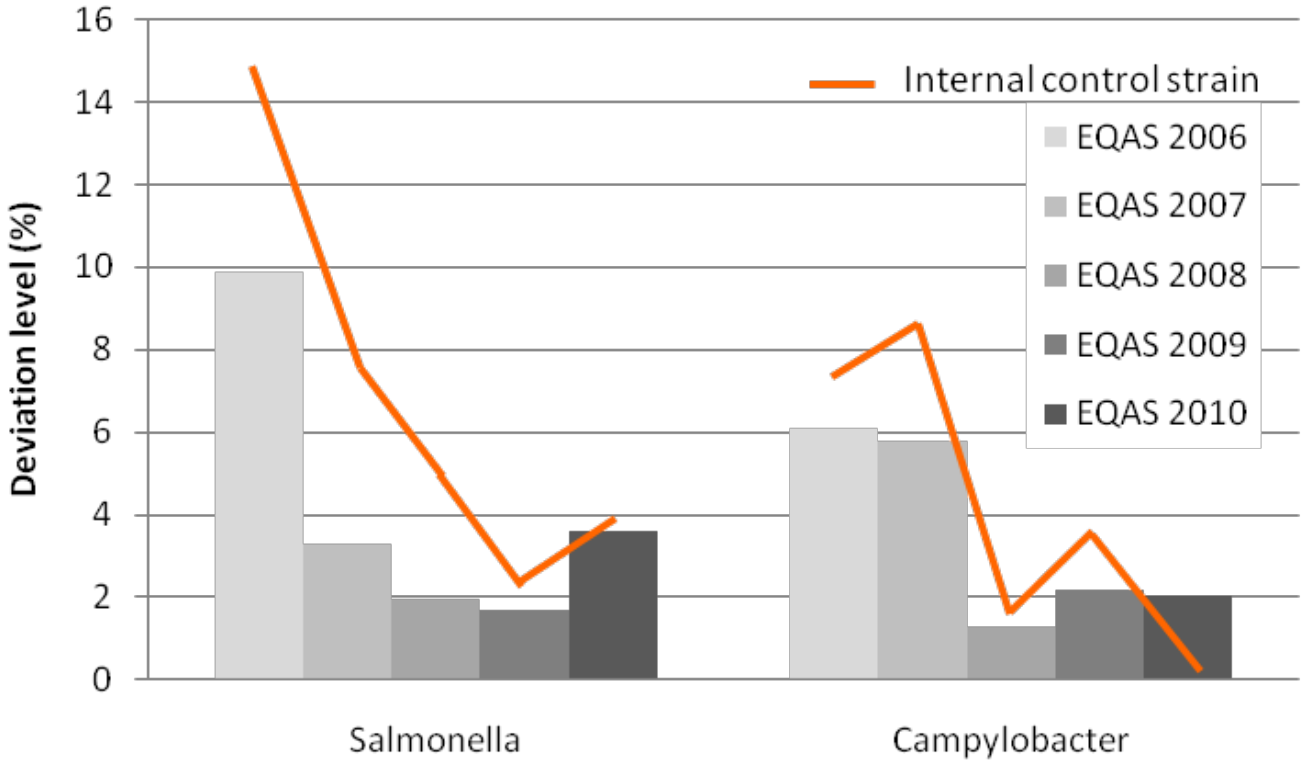
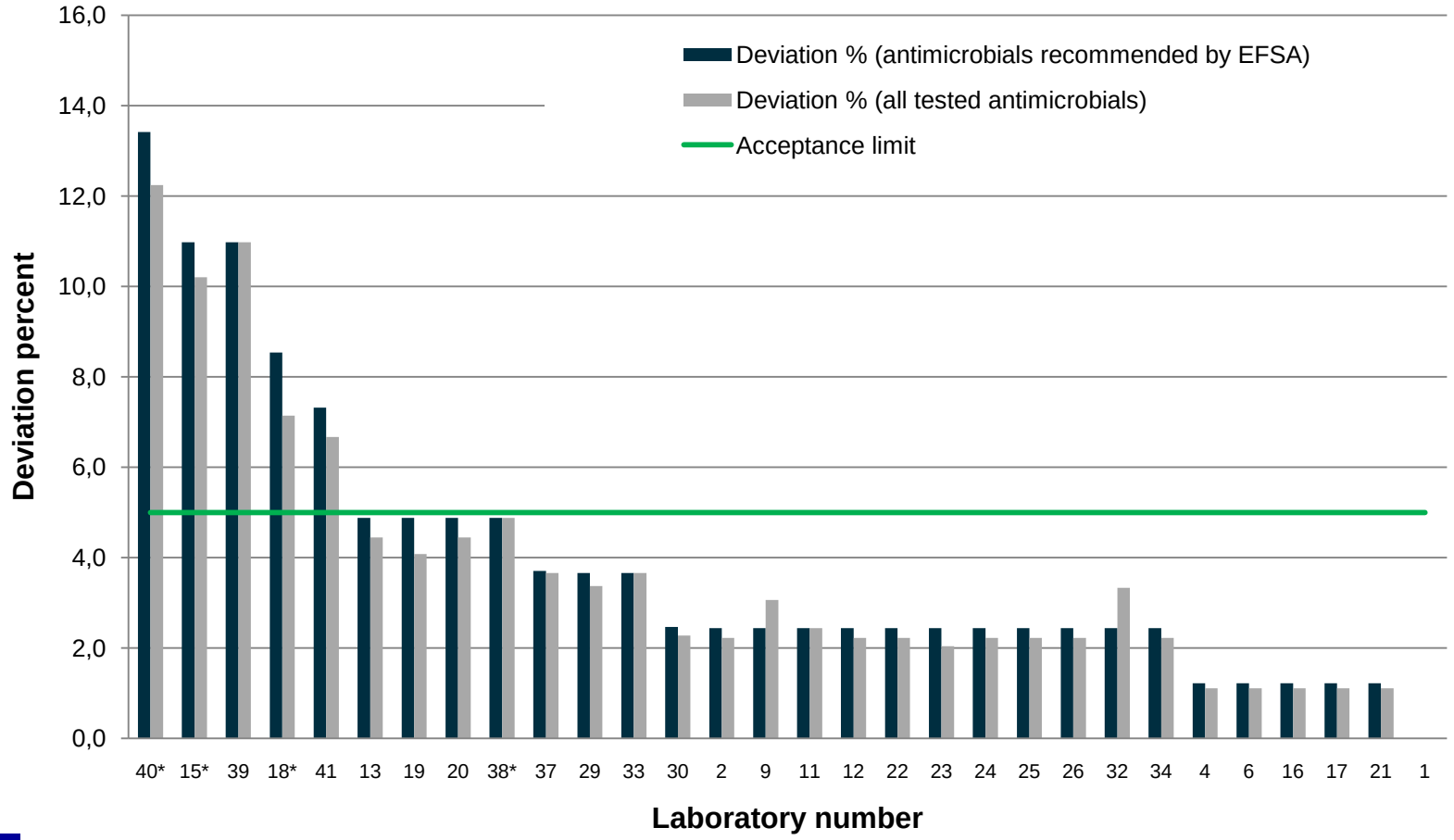
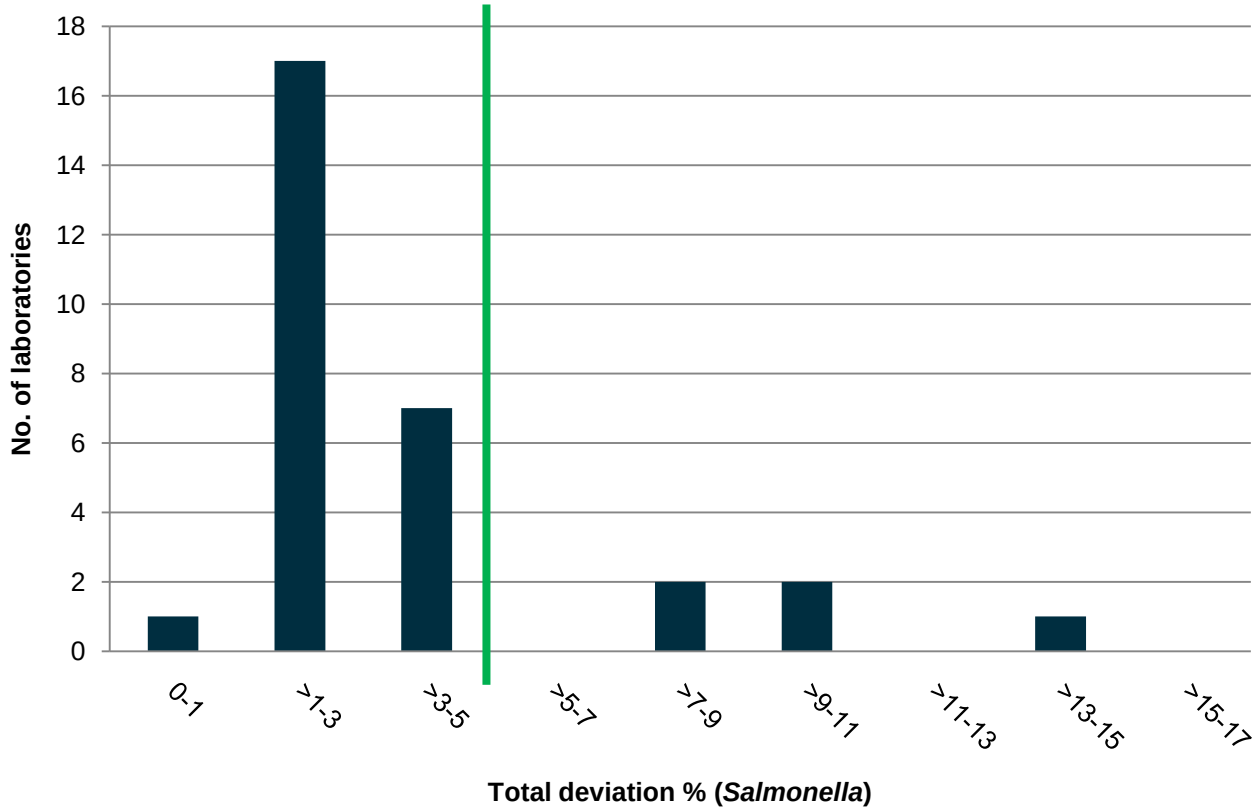
Comparison to former EQASs



Salmonella results – pr. lab



Salmonella results – intervals



Salmonella – deviations on antimicrobials

EQAS 2010	% correct
Ampicillin, AMP	99.6
Cefotaxime, CTX	99.6
Ceftazidime, CAZ	97.9
Ceftiofur, XNL	100.0
Chloramphenicol, CHL	99.2
Ciprofloxacin, CIP	90.8
Gentamicin, GEN	98.7
Nalidixic acid, NAL	95.8
Streptomycin, STR	81.7
Sulphonamides, SMX	98.3
Tetracycline, TET	98.3
Trimethoprim, TMP	100.0

CIP – towards *Salmonella* – cut off

- Ciprofloxacin (9.2% deviation; 22/240)
  - Low cut-off value is used: R>0.06µg/mL (lower than the CLSI clinical breakpoint)

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

!!! Word is, from June 2011:

- CLSI breakpoints for CIP/*Salmonella* will be lowered to
  - S=<0.064 ug/mL, I=0.125-0.5 and R=>=1 ug/mL
  - S=>31 mm; R<=20 mm
  - In June they plan to set breakpoints for other FQs for *Salmonella* and the *Enterobacteriaceae*

CIP – towards *Salmonella* – method

- Ciprofloxacin (9.2% deviation; 22/240)
  - Disk diffusion: 12/22 deviations
  - MIC-determination: 10/22 deviations
    Seven out of ten MIC's were actually > the cut off!

    - 5/22 deviations were on S-5.6 – harbouring *qnrS* (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**

QC strains – *Salmonella*, MIC

EQAS 2010 Antimicrobial	**MIC determination *E. coli* 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	0/26 (0%)	-	-
Cefotaxime, CTX	0/25 (0%)	-	-
Cefoxitin, FOX	0/4 (0%)	-	-
Ceftazidime, CAZ	0/19 (0%)	-	-
Ceftiofur, XNL	0/2 (0%)	-	-
Chloramphenicol, CHL	0/26 (0%)	-	-
Ciprofloxacin, CIP	2/25 (8%)	-	1 step
Gentamicin, GEN	0/26 (0%)	-	-
Nalidixic acid, NAL	0/25 (0%)	-	-
Streptomycin, STR	1/24 (4%)	1 step	-
Sulphonamides, SMX	0/16 (0%)	-	-
Tetracycline, TET	0/26 (0%)	-	-
Trimethoprim, TMP	0/24 (0%)	-	-

QC strains – *Salmonella*, disc diffusion

EQAS 2010 Antimicrobial	**Disk diffusion *E. coli* 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	1/4 (25%)	-	2
Cefotaxime, CTX	1/4 (25%)	-	1
Cefoxitin, FOX	0/4 (0%)	-	-
Ceftazidime, CAZ	0/3 (0%)	-	-
Ceftiofur, XNL	1/3 (33%)	2	-
Chloramphenicol, CHL	0/4 (0%)	-	-
Ciprofloxacin, CIP	0/4 (0%)	-	-
Gentamicin, GEN	0/4 (0%)	-	-
Imipenem, IMI	1/3 (33%)	-	3
Nalidixic acid, NAL	0/4 (0%)	-	-
Streptomycin, STR	0/4 (0%)	-	-
Sulphonamides, SMX	0/3 (0%)	-	-
Tetracycline, TET	0/4 (0%)	-	-
Trimethoprim, TMP	0/4 (0%)	-	-

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-5.7 (CTX M-15 like)	**Strain S-5.8 (CTX M-15)**
Confirmed ESBL	28/30 (93%)	27/30 (90%)
FOX^{S}	30/30 (100%)	30/30 (100%)
AmpC not confirmed	30/30 (100%)	30/30 (100%)

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

ESBL-producing test strains – detection

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

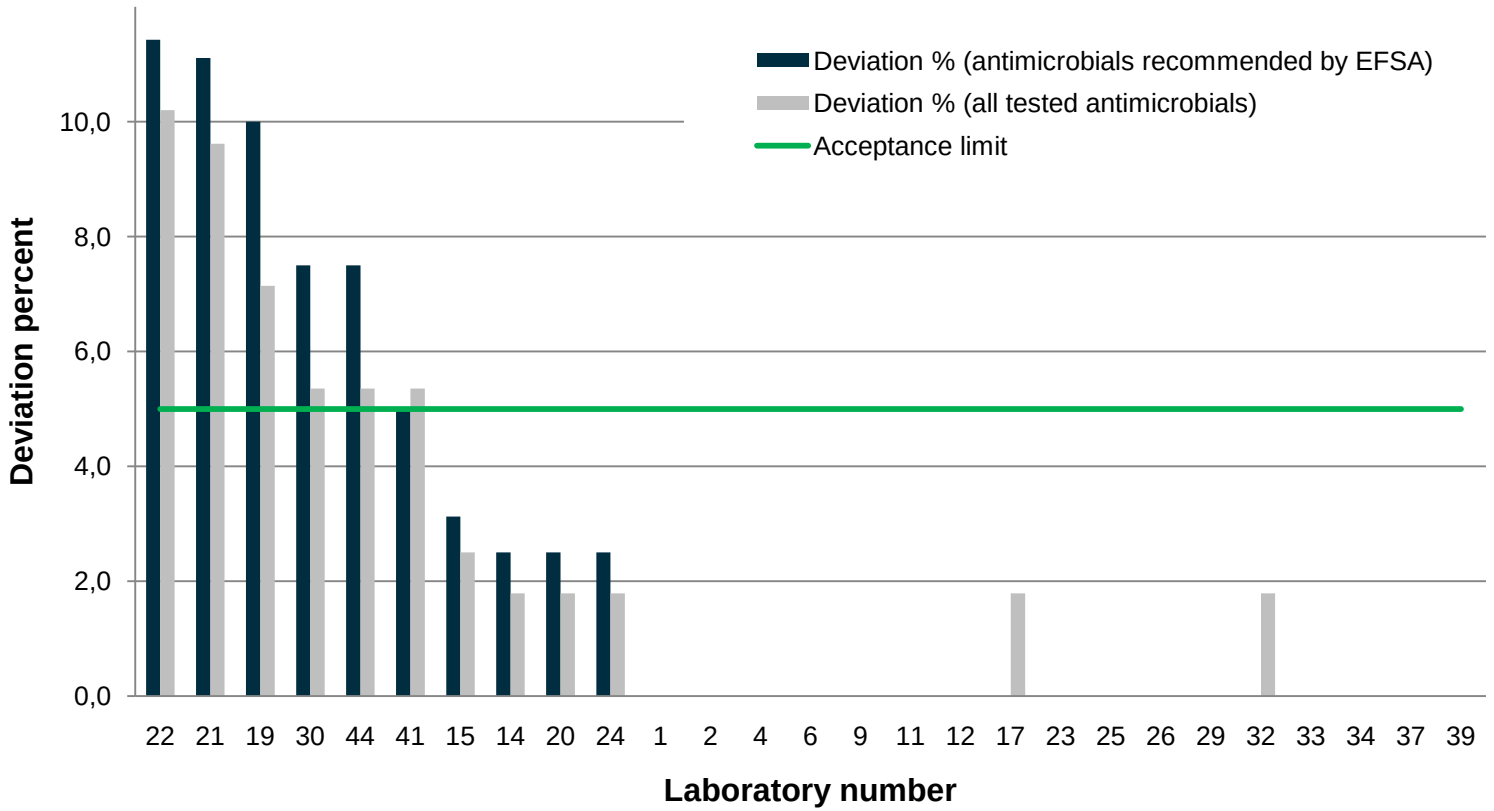
	Strain S-5.7 (CTX M-15 like)	Strain S-5.8 (CTX M-15)
CTX, CAZ, XNL	6/6 (100%)	6/6 (100%)
CTX, CAZ	18/18 (100%)	17/18 (94%)
CTX, XNL	1/1 (100%)	1/1 (100%)
CTX	3/5 (60%)	3/5 (60%)

=> EUCAST Expert rules: Concerning cefotaxime, ceftazidime and/or ceftiofur used when detecting ESBL-producing strains in this EQAS, MIC values and interpretations for these antimicrobials should be reported as found

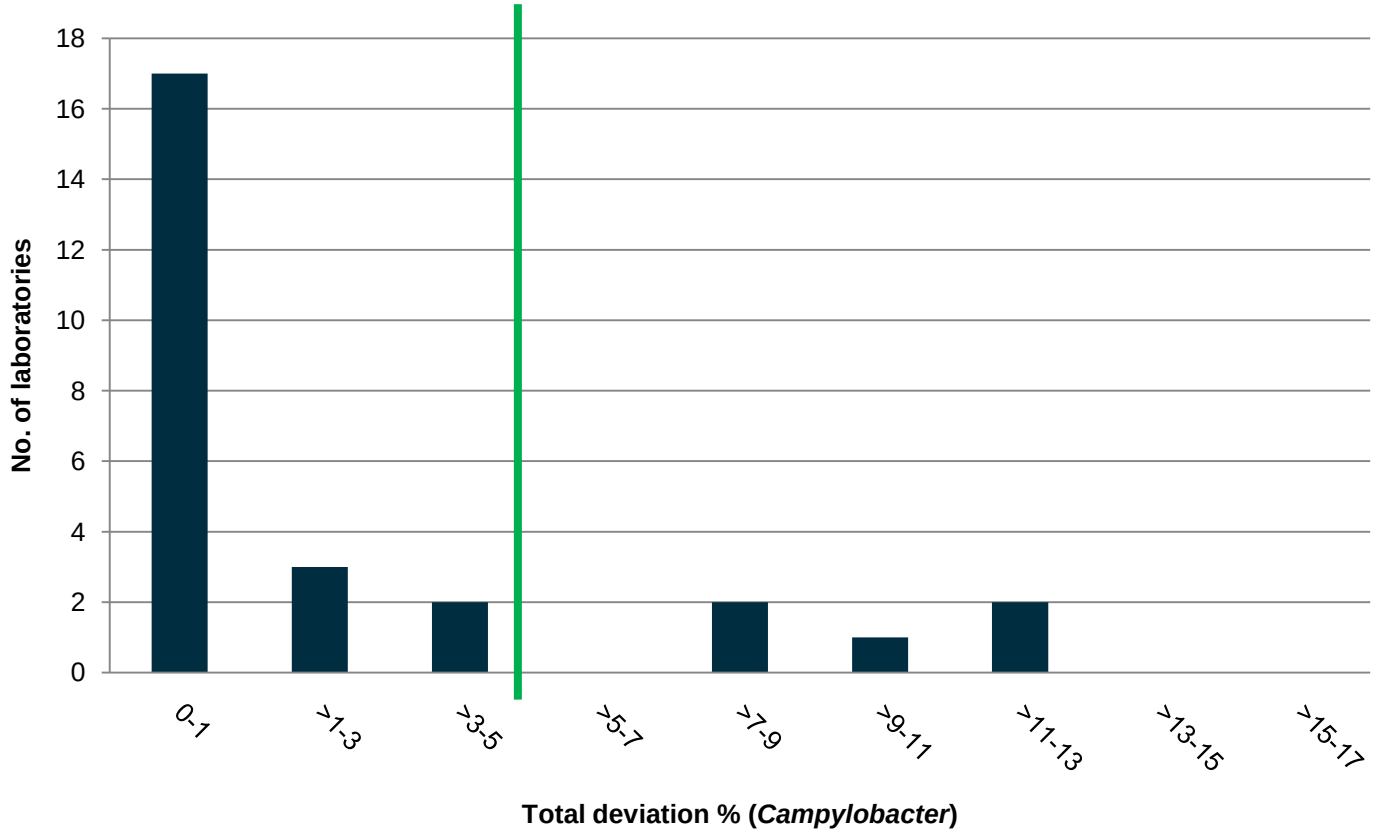
Campylobacter – deviations on antimicrobials

EQAS 2010	% correct
Chloramphenicol, CHL	100.0
Ciprofloxacin, CIP	99.1
Erythromycin, ERY	99.1
Gentamicin, GEN	100.0
Nalidixic acid, NAL	97.7
Streptomycin, STR	95.6
Tetracycline, TET	94.8

Campylobacter results – pr. lab



Campylobacter results – intervals



QC strains – *Campylobacter*, MIC

EQAS 2010 Antimicrobial	**MIC determination *C. jejuni* 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	0/16 (0%)	-	-
Ciprofloxacin, CIP	1/25 (4%)	-	3 steps
Erythromycin, ERY	2/25 (8%)	1 step	1 step
Gentamicin, GEN	4/18 (22%)	1 step	-
Nalidixic acid, NAL	0/22 (0%)	-	-
Tetracycline, TET	2/22 (9%)	-	1 step

- 25 out of 27 labs uploaded QC-strain data on Campy
- Proportion within the QC intervals: 2007: 83.8%
  2008: 89.2%
  2009: 93.3%
  2010: 93.2%

Genotypic characterisation - background

Strains

Enterococcus faecium

Shigella 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)

G-pos: AST-profile => conventional PCR
G-neg: AST-profile + microarray. Weak results were confirmed by PCR.

Verification of results

At US-FDA

Genotypic characterisation – GEN-2.1 (G-pos)

- Two laboratories participated
- All participating laboratories obtained satisfying results

		Lab I		Lab IV	
Aminoglycosides	aadE	1	in	1	in
Aminoglycosides	aph(3')-III	1	in	1	in
Glycopeptide	vanB	1	in	1	in
Macrolides	erm(B)	1	in	1	in
Penicillin	pbp5	1	in	1	in
Tetracycline	tet(L)	1	in	1	in
Tetracycline	tet(M)	1	in	1	in
Additional genes		tet(K)	in	tet(K)	in

Genotypic characterisation – GEN-2.2 (G-neg)

- Four laboratories participated
- All participating laboratories obtained satisfying results

	Lab I		Lab III		Lab IV		Lab V	
CTX-M-14	1/NT	in	1/1	P	1/NT	in	1/1	P
OXA-1	1/NT	in	1/1	P	1/NT	in	1/NT	P
OXA-30	1/NT	in	1/NT	P	1/NT	in	1/1	P
catA1	1	in	1	P	1	in	1	P
gyrA-83	NT		1/1	P	NT		1/1	P
parC-80	NT		1/1	P	NT		NT	
strA	1	in	1	P	1	in	1	P
strB	1	in	1	P	1	in	1	in
aadA	1	in	1	P	1	in	1	P
sul2	1	in	1	P	1	in	1	in
tetB	1	in	1	P	1	in	1	P
Groups detected	CTX-M-9	in			CTX-M-9	in		

Instant evaluation in the database

Accredited proficiency test => organisers must take reasonable precautions to prevent collusion between participants or falsification of results

- Not reasonable precaution to offer instant individual evaluation of results before deadline

Suggested action:

- That individual evaluation report is made available at deadline

Summing up I

The *Salmonella* trial

- 30 labs participated
- 25 labs performed with deviation levels < the acceptance level
- No outliers

Challenges:

Ciprofloxacin (disk diffusion)

Detection and confirmation of ESBL-producers

Summing up II

The *Campylobacter* trial

- 27 labs participated
- 22 labs performed with deviation levels < the acceptance level
- 17 labs had no deviations (EFSA antimicrobials)
- No outliers

Summing up III

The genotypic characterisation

- Good agreement with the expected
- Few participants

Thanks for your attention!

Thoughts? Questions?