EQA8-AST

External quality assessment of antimicrobial susceptibility testing for Salmonella and Campylobacter

The EQA team - Statens Serum Institut
ECDC

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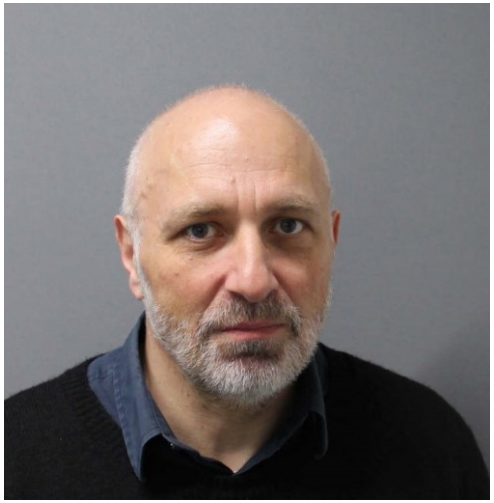

RefLabCap Mid term meeting,
Copenhagen, 26 April 2023

Objectives/aims of the EQA-AST's funded by ECDC

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Aims:

- support the implementation of the harmonized EU AST protocol
- assess the quality of the AST data obtained using MIC and/or DD methods in NPHRLs across Europe
- allow evaluation of new molecular based methodologies (WGS, PCR etc.)

Objectives:

- assess the overall comparability of routinely collected AST results from European NPHRLs

EQA8-AST 2022

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EQA8-AST for *Salmonella*

- Eight strains
- Five mandatory antimicrobials: Ampicillin, cefoxatime, meropenem, Cipro/pefloxacin and tetracycline
- Possible to report ESBL-, acquired AmpC-, and carbapenemase status of the test strains – both pheno- and genotypes
- Possible to report predicted results (WT or NWT) from molecular analysis
- Possible to report serotyping results

EQA8-AST for *Campylobacter*

- Five strains for AST testing and species determination
- Three mandatory antimicrobials, ampicillin, ciprofloxacin and tetracycline
- Gentamicin optional
- Possible to report predicted results (WT or NWT) from molecular analysis

Data analysis and evaluation

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- Test results were compared to the expected results from the EQA provider
  - *Salmonella*: MIC results within +/- one dilution difference and DD results within +/- 3 mm difference were evaluated as correct
  - *Campylobacter*: MIC results within +/- one dilution difference and DD results within +/- 4 mm difference were evaluated as correct
- MIC results that were not in the relevant concentration range for comparison with expected results were not evaluated (ND)
- Qualitative results interpreted using EUCAST ECOFF and clinical breakpoints
- ESBL/AmpC/carbapenemase pheno- and genotypic results evaluated case by case
- Predicted genotypic results evaluated against phenotypic qualitative results using ECOFF's

- Individual feed back have been provided to the participants

Salmonella
29 EU/EEA countries participated

Salmonella test strains EQA8 AST

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Strain	Serotype	Microbiological resistance profile* (NWT)	Genotype, selected resistance genes
EQA_AST.S22.0001	Monophasic Typhimurium, 4,12:i:-	AMP, CHL, TCY, TMP, TMP-SMX	
EQA_AST.S22.0002	Monophasic Typhimurium, 4,12:i:-	AMP, CHL, GEN, NAL, TCY	*blaCTX-M-55*
EQA_AST.S22.0003	Monophasic Typhimurium, 4,5,12:i:-	AMP, CHL, GEN, NAL, TCY, TMP TMP-SMX	
EQA_AST.S22.0004	Monophasic Typhimurium, 4,5,12:i:-	AMP, CAZ, CHL, CIP, CTX, FEP, GEN, NAL, PEF, TCY	*blaCTX-M-55*
EQA_AST.S22.0005	Heidelberg	AMP, AZM, CAZ, CHL, CIP, CTX, FEP, PEF, TCY, TMP, TMP-SMX	*blaCTX-M-123*
EQA_AST.S22.0006	Monophasic Typhimurium, 4,5,12:i:-	AMP, FEP, FOX, MEM, TCY	
EQA_AST.S22.0007	Newport	AMP, CAZ, CHL, CTX, FEP, FOX, TCY, TMP-SMX	*blaCMY-2*
EQA_AST.S22.0008	Senftenberg	AMI, AMP, CAZ, CEP, CIP, CTX, FOX, GEN, MEM, NAL, PEF	*blaNDM-1, blaSHV-12, blaCMY-4*

EQA8-AST SALMONELLA – OVERALL RESULTS

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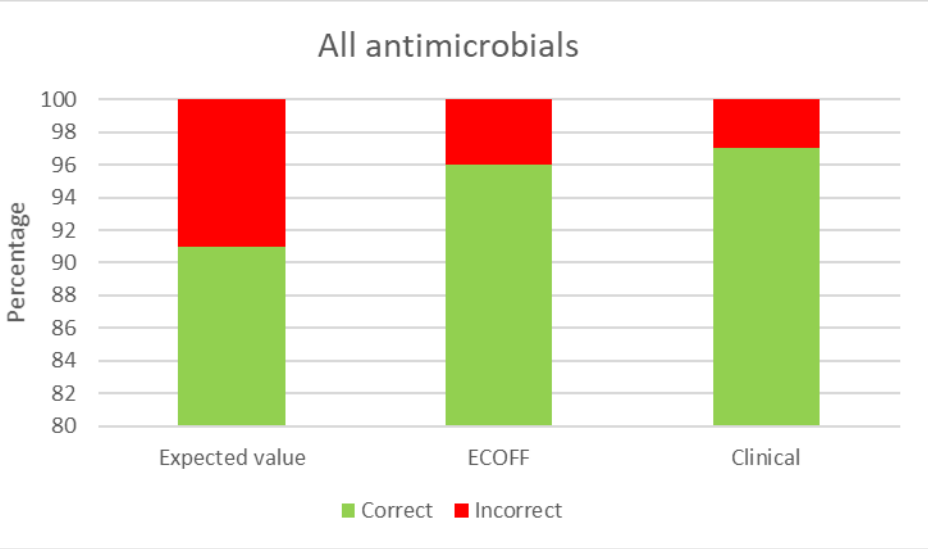
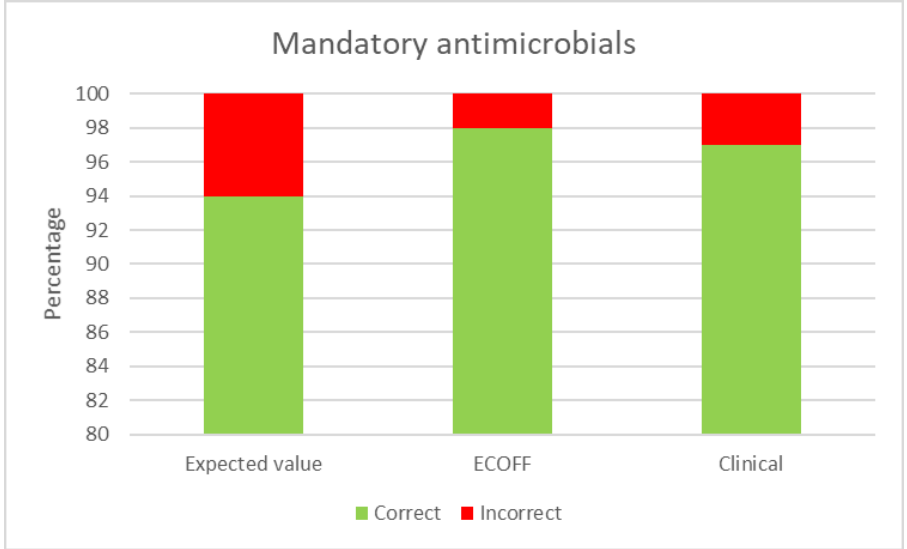
DD and MIC results evaluated against expected quantitative and expected qualitative results using ECOFF's and clinical breakpoints

Results by DD assay	All antimicrobials	Mandatory	Optional
Expected value	1703/1869 (91%)	651/693 (94%)	1052/1176 (89%)
ECOFF	1572/1637 (96%)	680/693 (98%)	892/944 (94%)
Clinical	1313/1352 (97%)	537/552 (97%)	776/800 (97%)
NA (ECOFF/clinical breakpoint)	232/517	0/141	232/376
NI	80	40	40
total	1949		
Results by MIC determination	**All antimicrobials**	**Mandatory**	**Optional**
Expected value	1437/1504 (96%)	486/507 (96%)	951/997 (95%)
ECOFF breakpoints	1133/1200 (94%)	498/525 (95%)	635/675 (94%)
Clinical breakpoints*	971/1020 (95%)	410/428 (96%)	561/592 (95%)
NA (ECOFF/clinical breakpoint)	336/545	0/97	366/448
ND	61	18	43
NI	29	22	7
Total	1594		

NA: Not analyzed, no EUCAST breakpoints
ND: MIC results that were not in the relevant range for comparison with expected results
NI: Not included for analysis, either because the disk concentration used deviated from the recommended (DD) or that the range tested did not cover the ECOFF breakpoint (MIC)

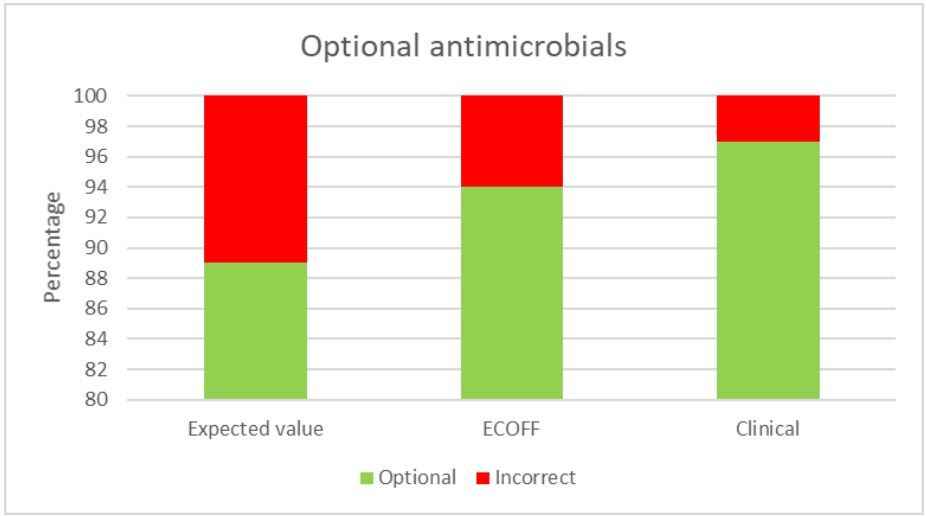
QUANTITATIVE SALMONELLA DD RESULTS COMPARED WITH EQA PROVIDERS RESULTS



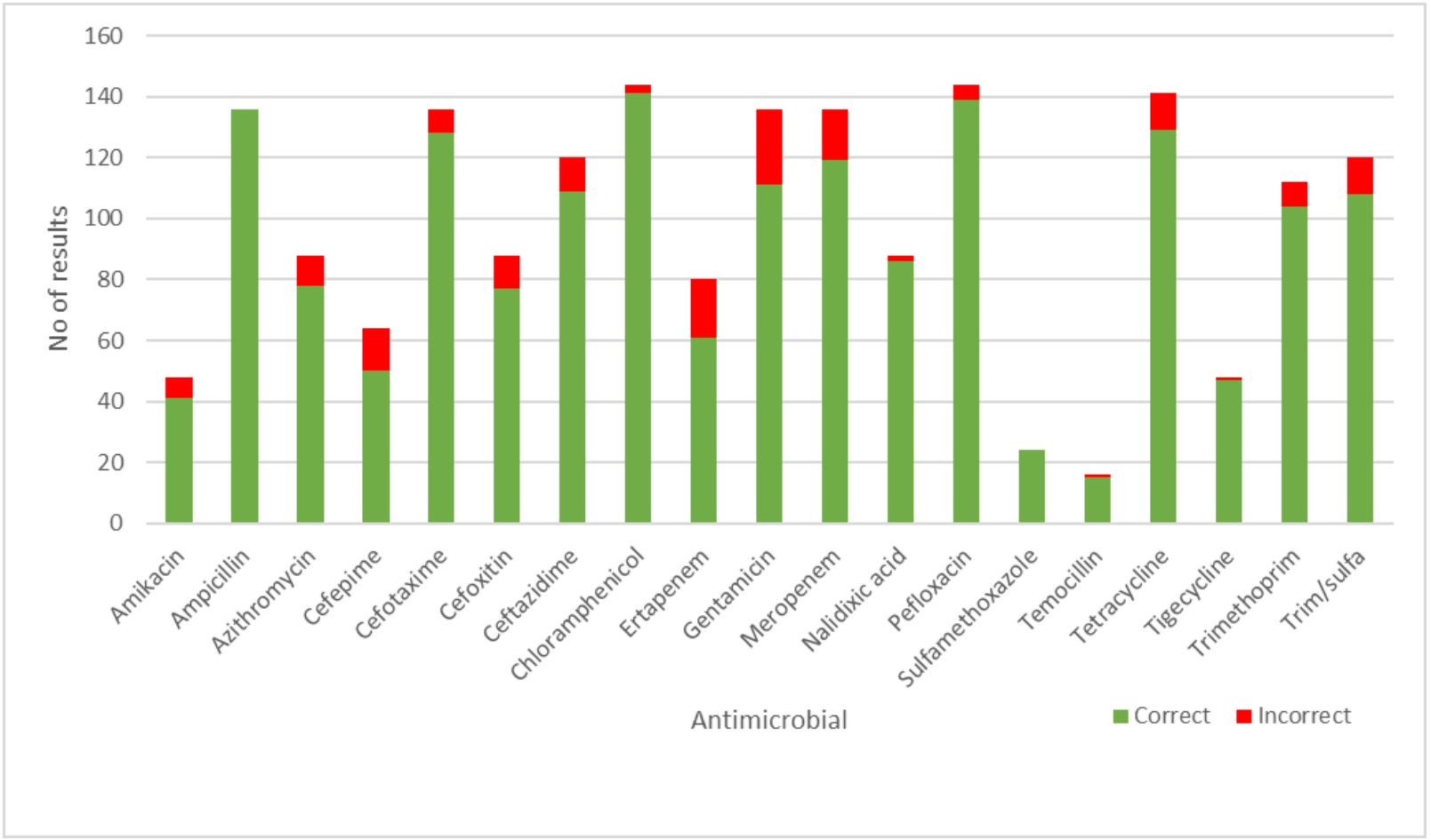


Mandatory antimicrobials:

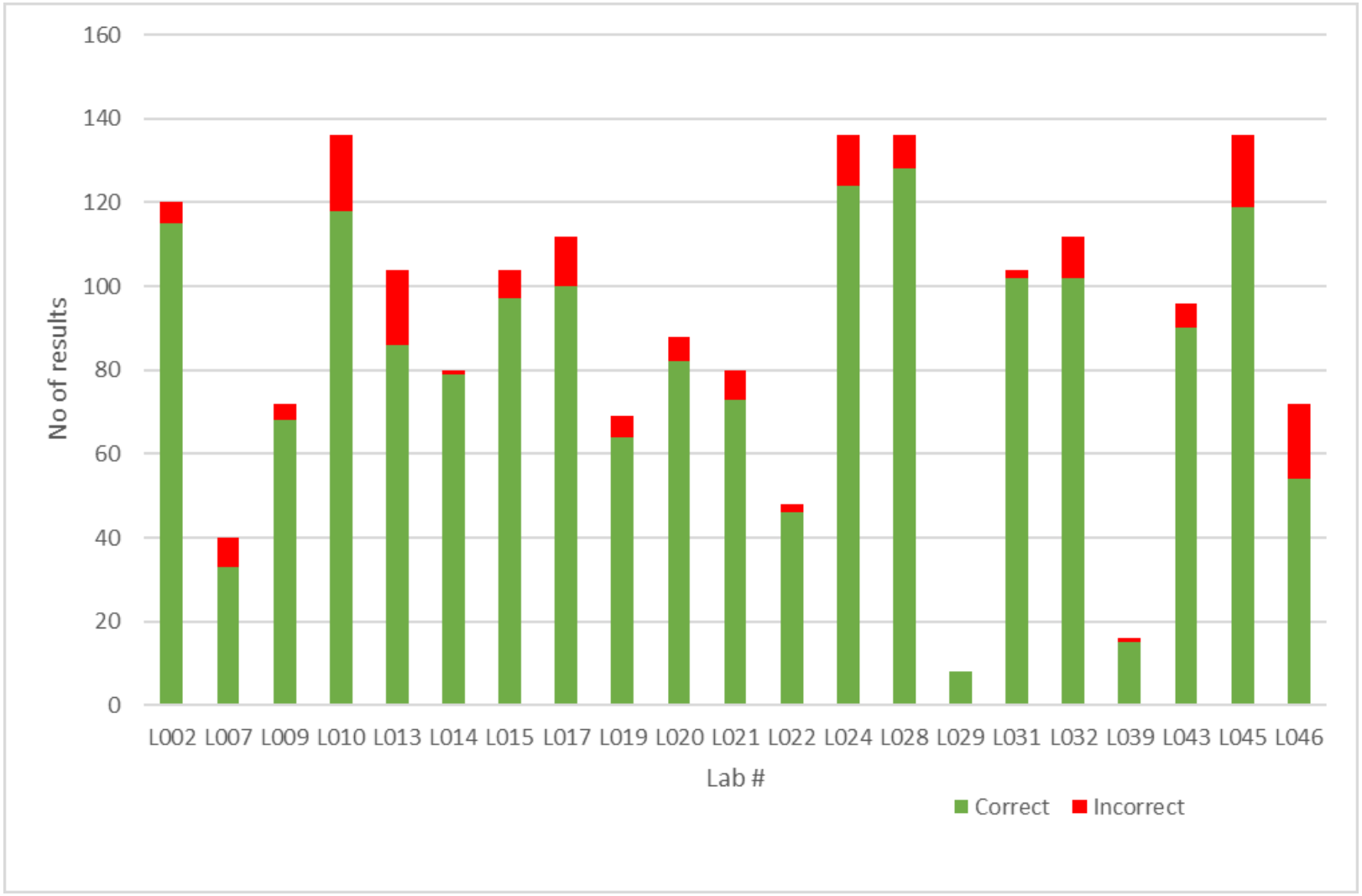
- Ampicillin
- Cefotaxime
- Meropenem
- Pefloxacin
- Tetracycline



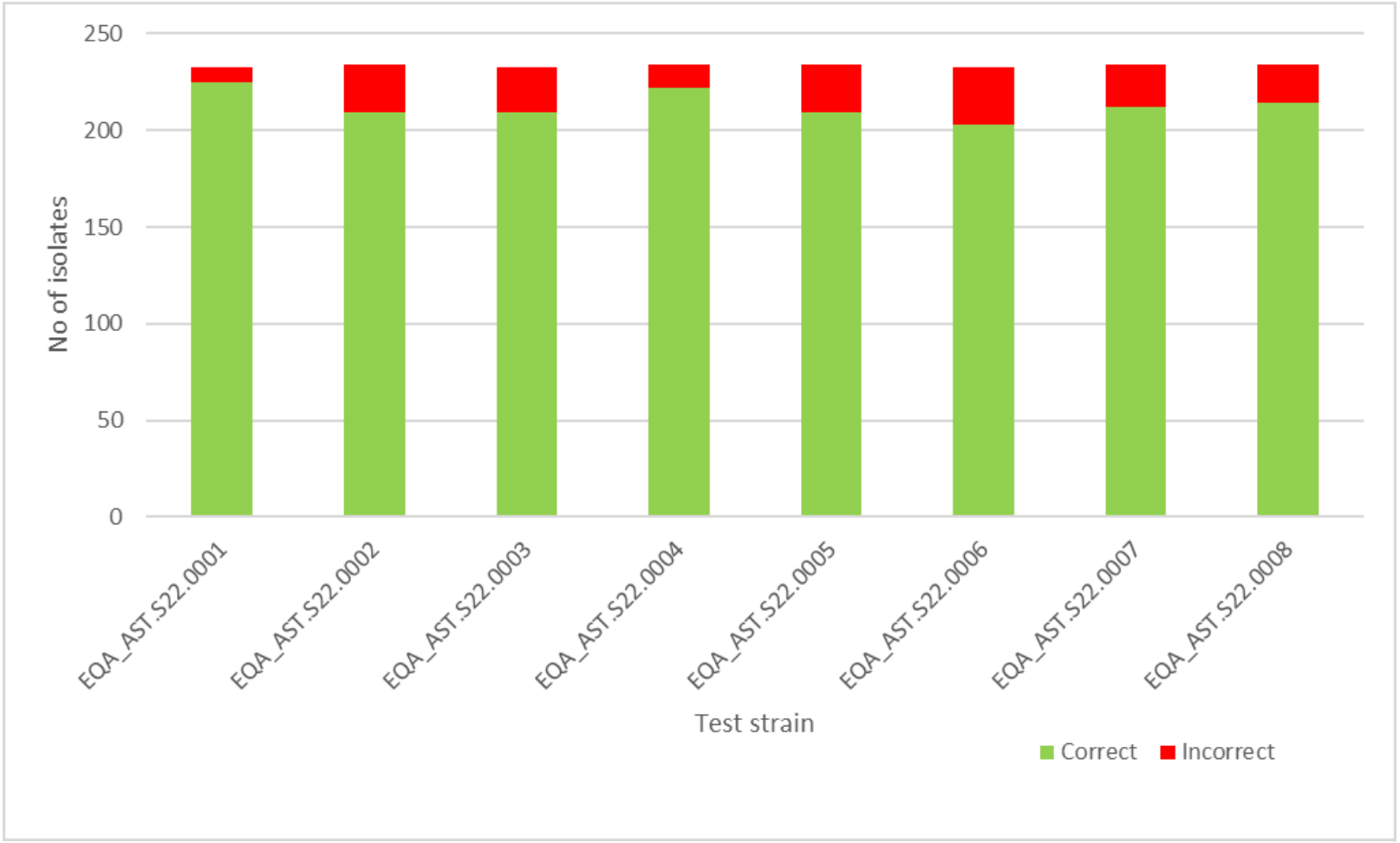
Salmonella: Quantitative DD results – all antimicrobials



Salmonella: Quantitative results DD – by laboratory -all antimicrobials



Salmonella: Quantitative DD results by test strain - all antimicrobials



QUANTITATIVE SALMONELLA MIC RESULTS COMPARED WITH EQA PROVIDERS RESULTS

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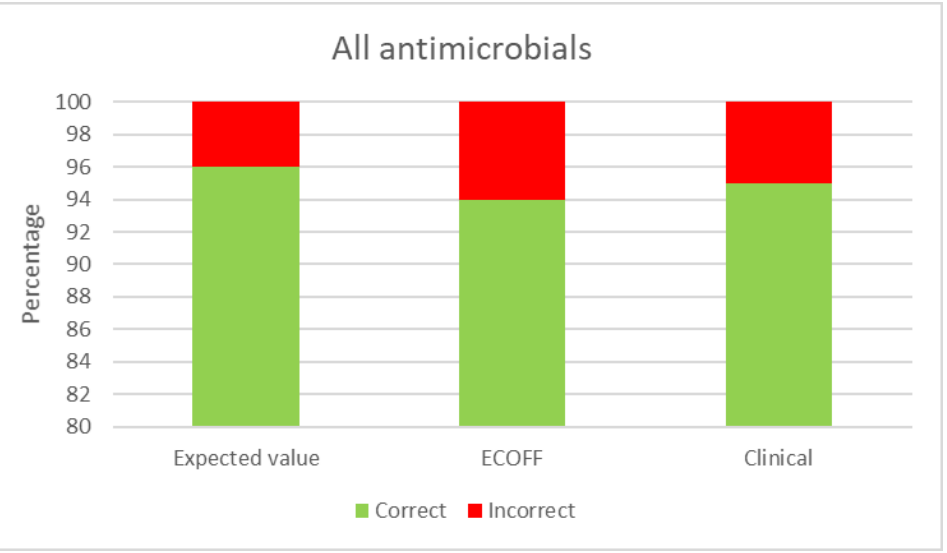
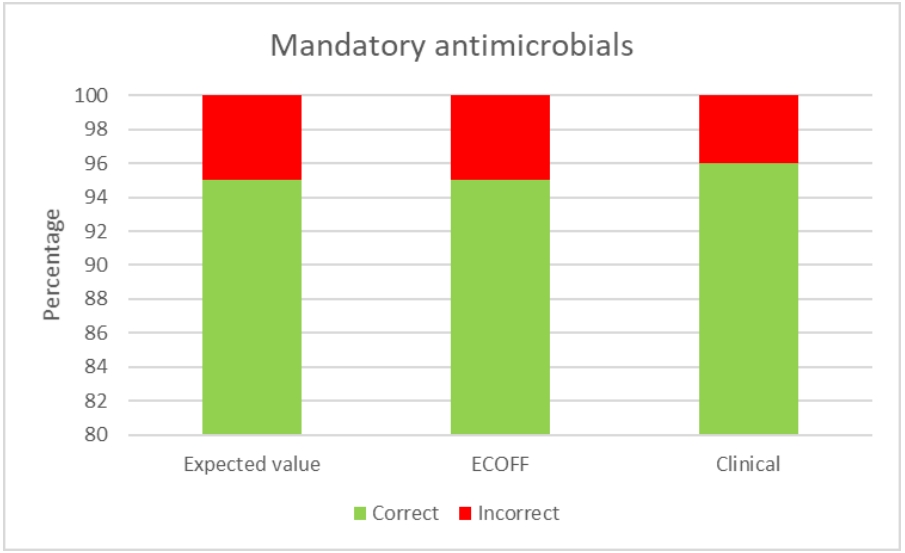
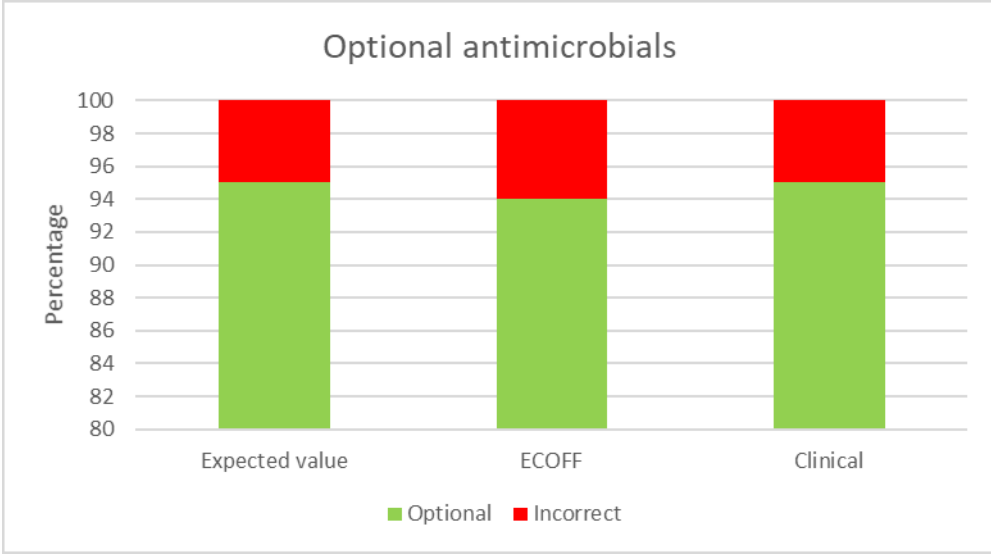




Mandatory antimicrobials

- Ampicillin
- Cefotaxime
- Meropenem
- Ciprofloxacin
- Tetracycline



Salmonella: quantitative MIC results – antimicrobials and methods

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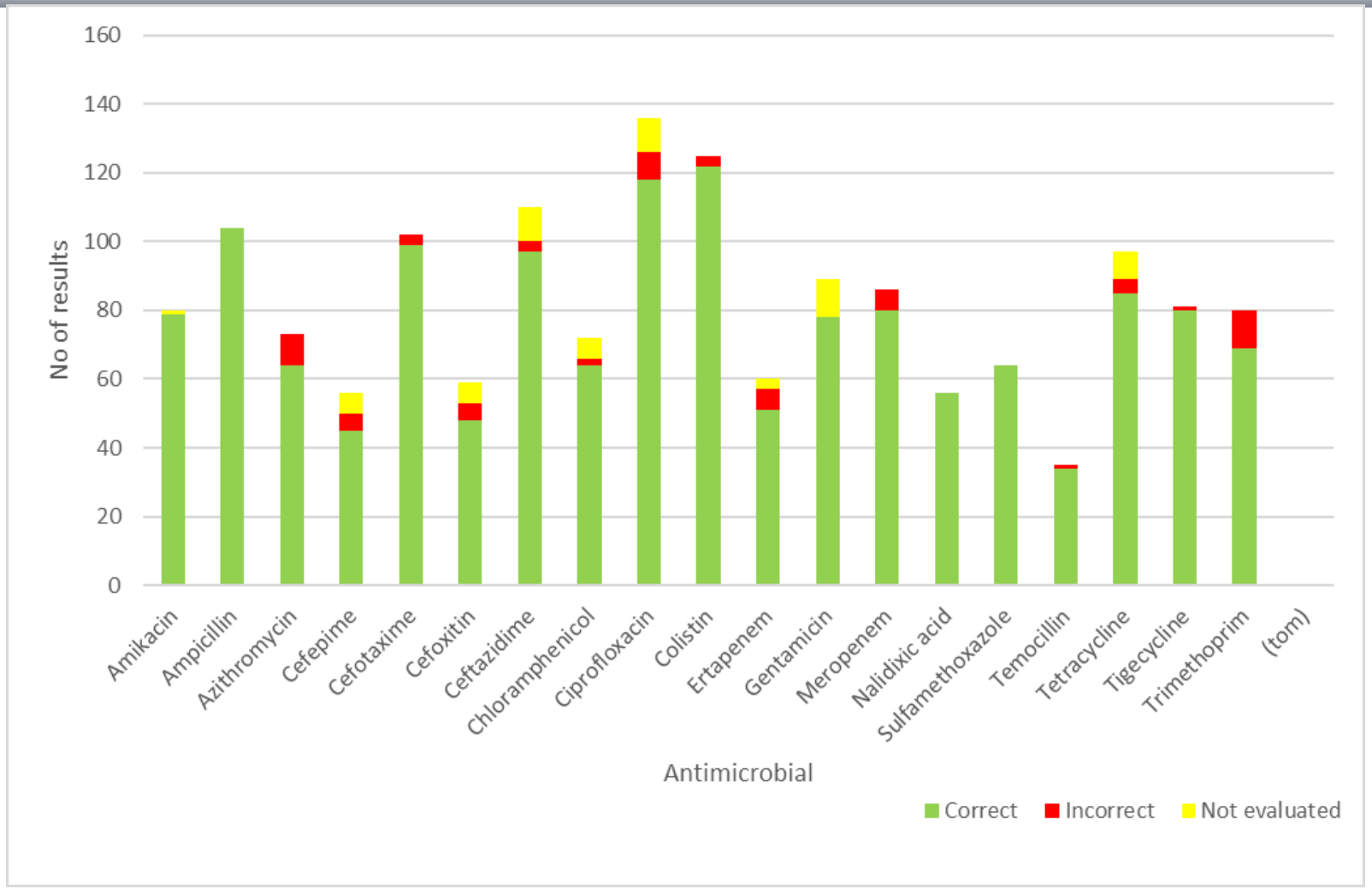

1594 results
1500 Broth dilution
94 Gradient strip methods

Most ND-results: correct ECOFF interpretation

Salmonella: Quantitative MIC results – by laboratories
All antimicrobials

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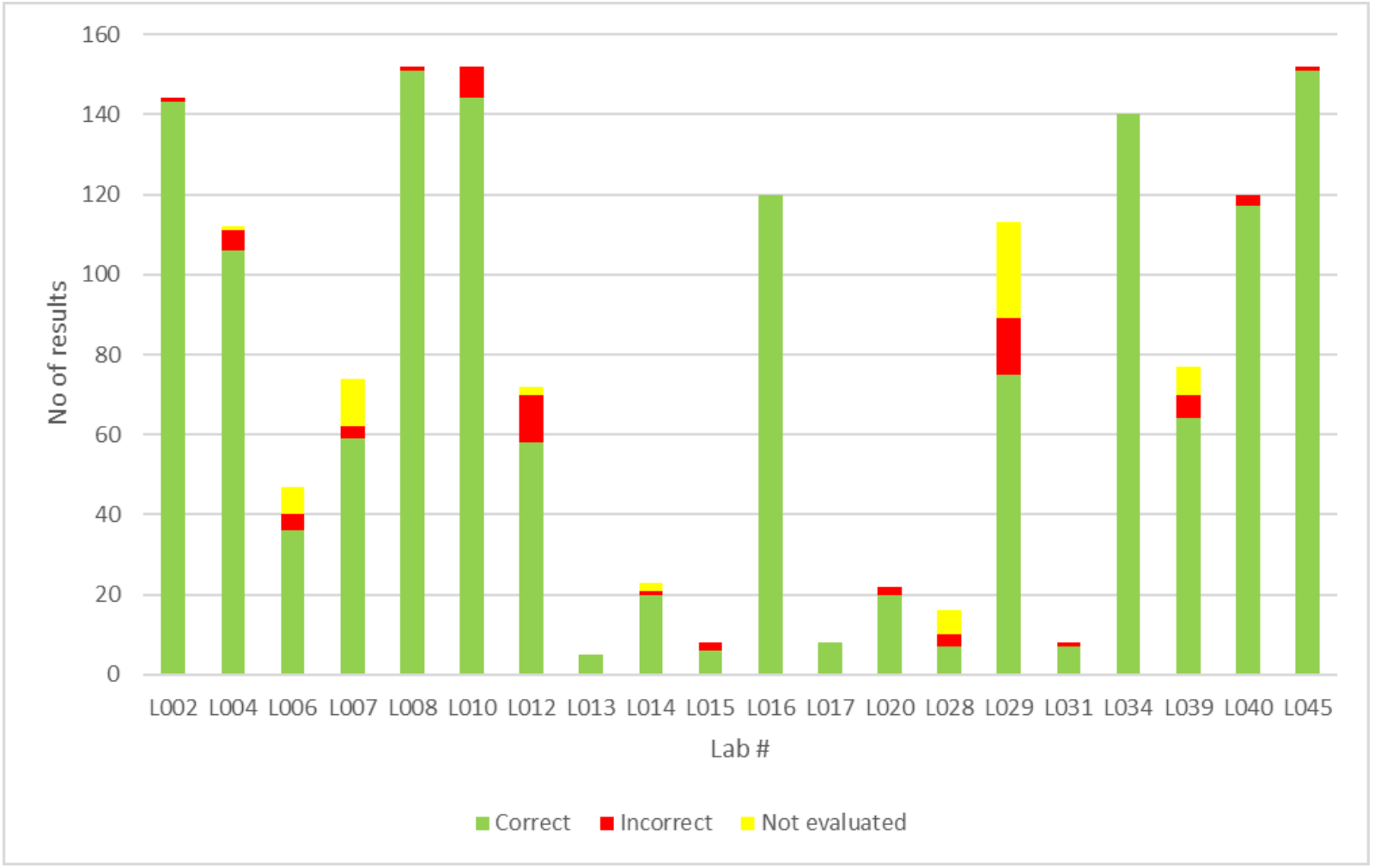
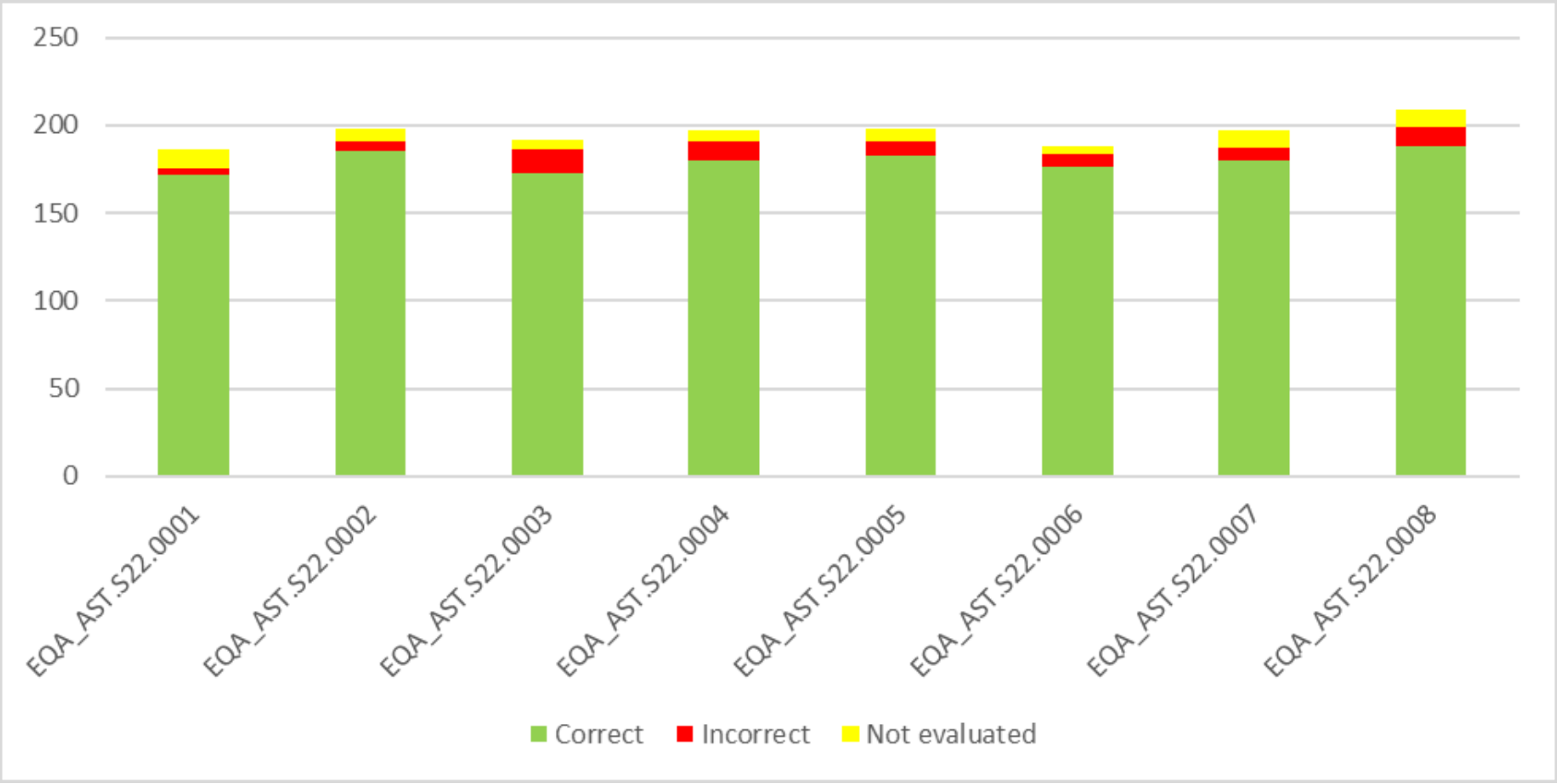


Salmonella: Quantitative MIC results – by strains
All antimicrobials



Phenotypic prediction of ESBL-, acquired AmpC and carbapenemase-production

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Strain	AmpC	ESBL	Carbapenemase
EQA_AST.S22.0001			
EQA_AST.S22.0002	1	18	1
EQA_AST.S22.0003			
EQA_AST.S22.0004		20	1
EQA_AST.S22.0005		21	1
EQA_AST.S22.0006			
EQA_AST.S22.0007	19	1	
EQA_AST.S22.0008	6	7	20
Total	27	68	23

Some of the phenotypes could not entirely be justified from the reported data

Genotypic characterization of ESBL-, acquired AmpC, and carbapenemase genes

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Strain	Expected genotype	Method used for genotype prediction	Genotype predicted	No of laboratories
S22.0002	blaCTX-M-55	WGS	blaCTX-M-55	8
		PCR + WGS	blaCTX-M-55	1
		PCR+ sequencing	blaCTX-M-55	1
		PCR	blaCTX-M	2
		PCR	CTX-M-1	2
		PCR	blaCTX-1	1
		PCR	CTX-M-1	1
	Total			16
S22.0004	*bla* CTX-M-55	WGS	blaCTX-M-55	11
		PCR + WGS	blaCTX-M-55	1
		PCR+ sequencing	blaCTX-M-55	1
		PCR	blaCTX-M	1
		PCR	blaCTX-M-1	1
		PCR	blaCTX-M-55	1
		PCR	blaCTX-M-1	1
	Total			17
S22.0005	blaCTX-M-123	WGS	blaCTX-M-123	13
		PCR + WGS	blaCTX-M-123	1
		PCR+ sequencing	blaCTX-M-123	1
		PCR	blaCTX-M-123	1
		PCR	blaCTX-M	1
		PCR	blaCTX-M-9	1
	Total			18
S22.0007	blaCMY-2	WGS	blaCMY-2	9
		PCR + WGS	blaCMY-2	1
		PCR+ sequencing	blaCMY-2	2
		PCR	blaCMY-2	2
		PCR	blaCMY	1
	Total			15
S22.0008	blaNDM-1,blaSHV-12,blaCMY-4	WGS	blaNDM-1, blaSHV-12, blaCMY-4	9
			blaNDM-1, blaCMY-4	1
			blaNMD	1
		PCR + WGS	blaNDM-1, blaCMY-4	1
		PCR+ sequencing	blaNDM-1, blaCMY-4	1
		PCR	blaNMD, blaCMY2	1
		PCR	blaNMD, blaSHV	1
		PCR	blaNMD	6
	Total			21
Grand total				87

87 results reported for the five eligible strains

Generally the laboratories were able to identify the correct genes

Results reflects the lack of a standardized nomenclature

Results reported in different “forms” – some curation were done

SALMONELLA PREDICTED PHENOTYPES FROM WGS

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Predicted phenotypes from WGS data by antimicrobial					
Antimicrobial	**Correct**	**Incorrect**	**Incorrect NWT**	**Incorrect WT**	**Correct, total**
Amikacin	36	40	39	1	36/76 (47%)
Ampicillin	85				85/85 (100%)
Azithromycin	73	2		2	73/75 (97%)
Cefepime	60	16		16	60/76 (79%)
Cefotaxime	84	1	1		84/85 (99%)
Cefoxitin	56	19	1	18	56/75 (75%)
Ceftazidime	75	1	1		75/76 (99%)
Chloramphenicol	66	1		1	66/67 (99%)
Ciprofloxacin	82	1	1		82/83 (99%)
Colistin	72	3	3		72/75 (96%)
Ertapenem	67				67/67 (100%)
Gentamicin	75				75/75 (100%)
Meropenem	73	10		10	73/83 (88%)
Nalidixic acid	57	10	2	8	57/67 (85%)
Sulfamethoxazole	70	5		5	70/75 (93%)
Tetracycline	80	3		3	80/83 (96%)
Trimethoprim	75				
Total	1186	112	48/659 (7%)	64/639 (10	

91% of the reported results (1298) were correctly predicted as WT/NWT

Amikacin – caused problems – only one strain phenotypically resistant

SALMONELLA PREDICTED PHENOTYPES FROM WGS

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Predicted phenotype from WGS data by strain			
Strain	**Correct**	**Incorrect**	Total
EQA_AST.S22.0001	150	6	150/156 (96%)
EQA_AST.S22.0002	144	15	144/159 (91%)
EQA_AST.S22.0003	138	17	138/155 (89%)
EQA_AST.S22.0004	164	8	164/172 (95%)
EQA_AST.S22.0005	159	13	159/172 (92%)
EQA_AST.S22.0006	120	35	120/155 (77%)
EQA_AST.S22.0007	140	17	140/157 (89%)
EQA_AST.S22.0008	171	1	171/172 (99%)
Total	1186	112	1186/1298 (91%)

Predicted phenotypes from WGS data by laboratory			
Laboratory no	**Correct**	**Incorrect**	**Total**
L002	122	14	122/136 (90%)
L004	39	2	39/41 (95%)
L009	121	15	121/136 (89%)
L010	117	11	117/128 (91%)
L014	54	3	54/57 (95%)
L016	122	6	122/128 (95%)
L017	123	13	123/136 (90%)
L022	123	13	123/136 (90%)
L032	117	11	117/128 (91%)
L033	129	7	129/136 (95%)
L045	119	17	119/136 (88%)
Total	1186	112	1186/1298 (91%)

Conclusions *Salmonella*

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- EQA8 – highest participation ever recorded
- Good correspondence between expected and reported results
- A few laboratories - issues with the control strain ATCC 25922
- Most laboratories indicated correct phenotypic results for ESBL-, acquired AmpC and carbapenemase-production
- Laboratories submitted correct genotypic results for ESBL-, acquired AmpC and carbapenemase-production
- Results in line with results from previous EQA-AST's
- Eleven laboratories used WGS to predict resistance – with a fair degree of success
- No common laboratory problem identified
- **Results indicate that it is possible to compare phenotypic DD and MIC AST Salmonella results from NPHRLs across Europe**

Campylobacter –

25 EU/EEA countries participated

Campylobacter test strains by species and resistance profile

Strain	Species	Resistance profile[1] (NWT)
EQA8_AST.C22.0001	***C. jejuni***	**Ciprofloxacin, Gentamicin, Tetracycline**
EQA8_AST.C22.0002	***C. coli***	**Ciprofloxacin, Tetracycline**
EQA8_AST.C22.0003	***C. jejuni***	**Ciprofloxacin, Tetracycline**
EQA8_AST.C22.0004	***C. coli***	**Ciprofloxacin, Gentamicin, Tetracycline**
EQA8_AST.C22.0005	***C. coli***	**Ciprofloxacin, Erythromycin, Tetracycline**

- All laboratories, exept one reported correct species

EQA8-AST CAMPYLOBACTER – OVERALL RESULTS

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Results by DD	All antimicrobials	Mandatory	Gentamicin (Optional)
Expected value	234/285 (82%)	205/240 (85%)	29/45 (64%)
ECOFF	271/285 (95%)	229/240 (95%)	42/45 (93%)
Clinical breakpoint	231/240 (96%)	231/240 (96%)	
NA - No clinical breakpoint	45		45
Total	285	240	45
Results by MIC determination	**All antimicrobials**	**Mandatory**	**Optional**
Expected value	174/204 (75%)	134/154 (74%)	40/50 (77%)
ND	29	27	2
ECOFF	223/233 (96%)	174/181 (96%)	49/52 (94%)
Clinical breakpoint	171/181 (96%)	171/181 (96%)	
NA - No clinical breakpoint			52
Total	233	181	52
Results by WGS (predicted)	**All antimicrobials**	**Mandatory**	**Optional**
ECOFF	152/159 (96%)	117/123 (95%)	35/36 (97%)

CAMPYLOBACTER QUANTITATIVE AND QUALITATIVE DD AND MIC RESULTS

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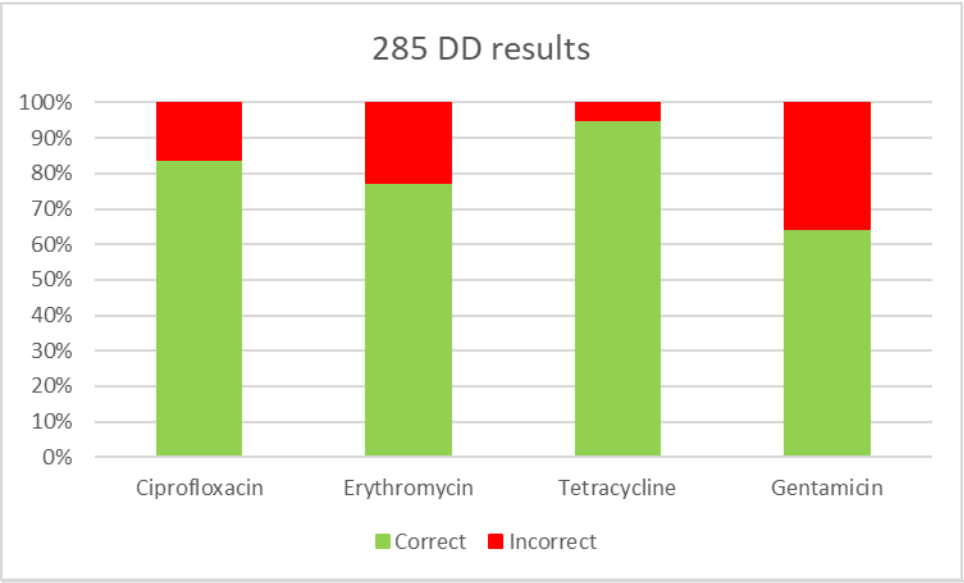
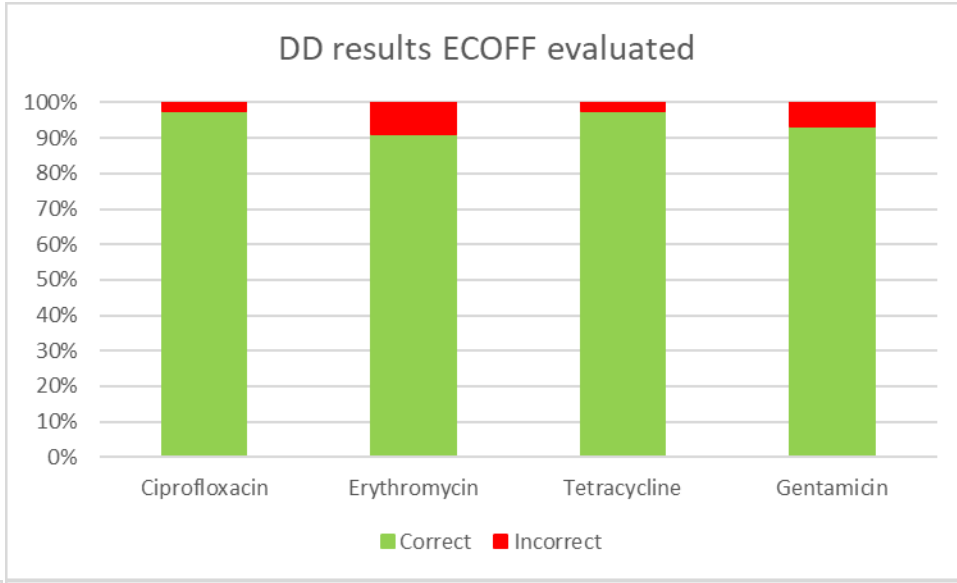
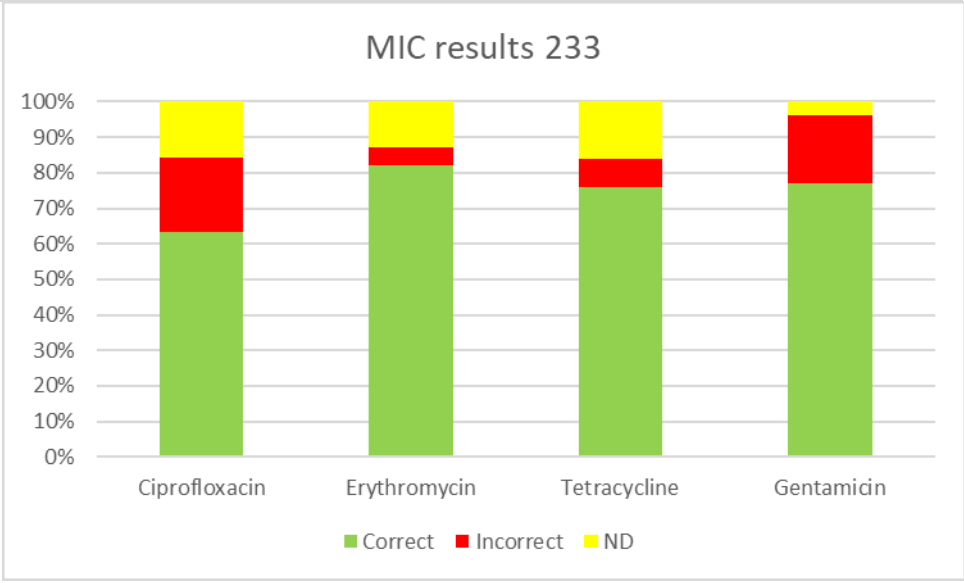
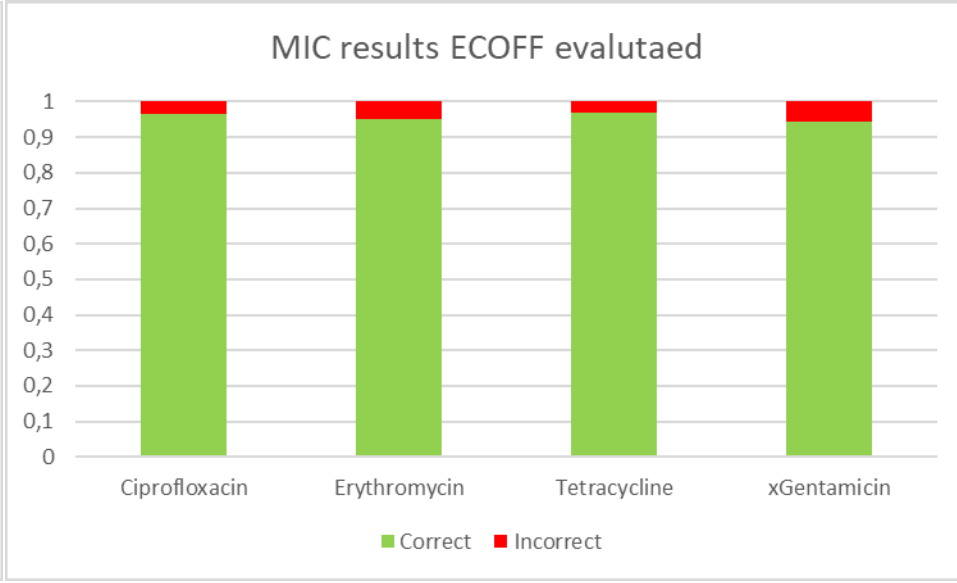
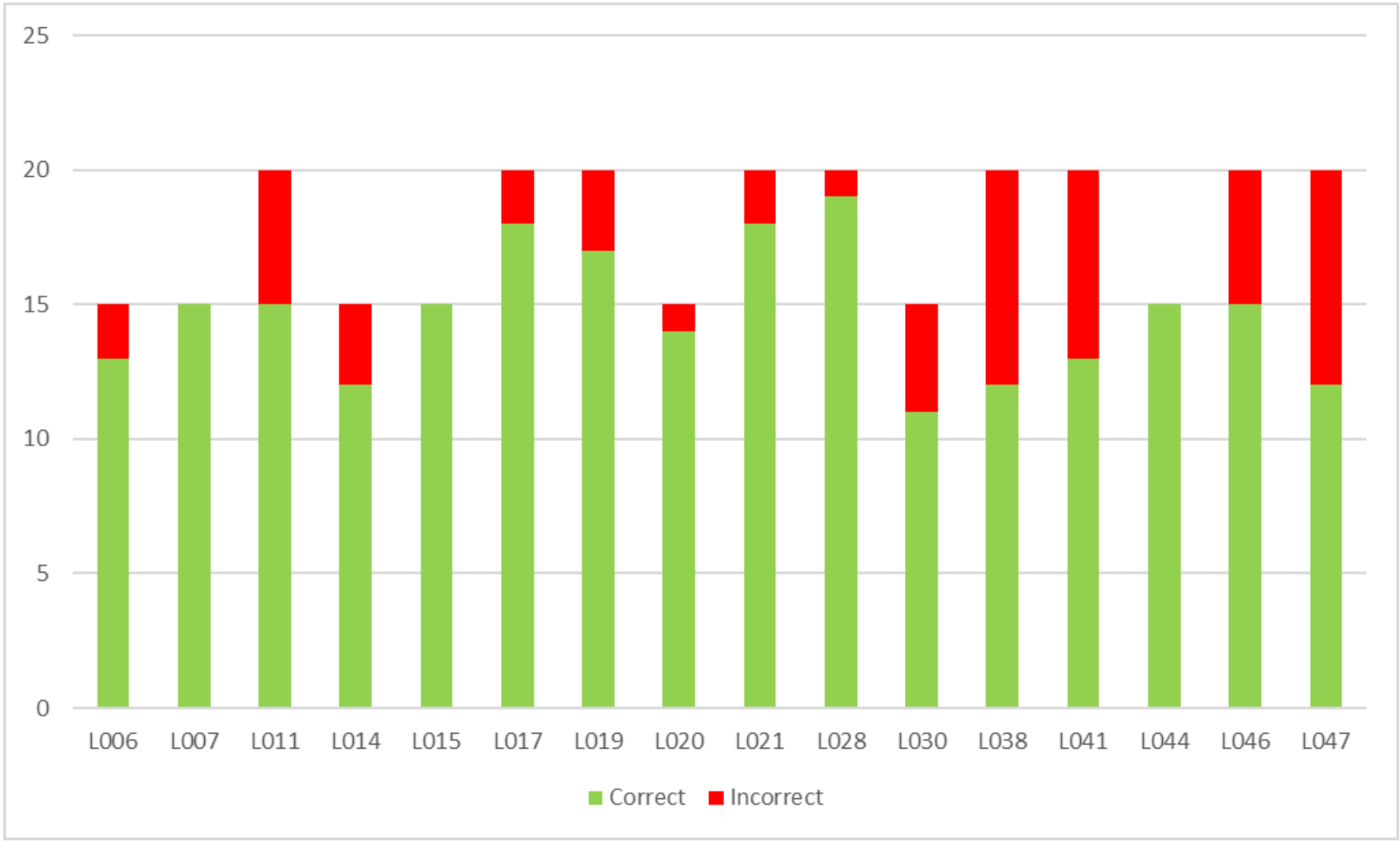






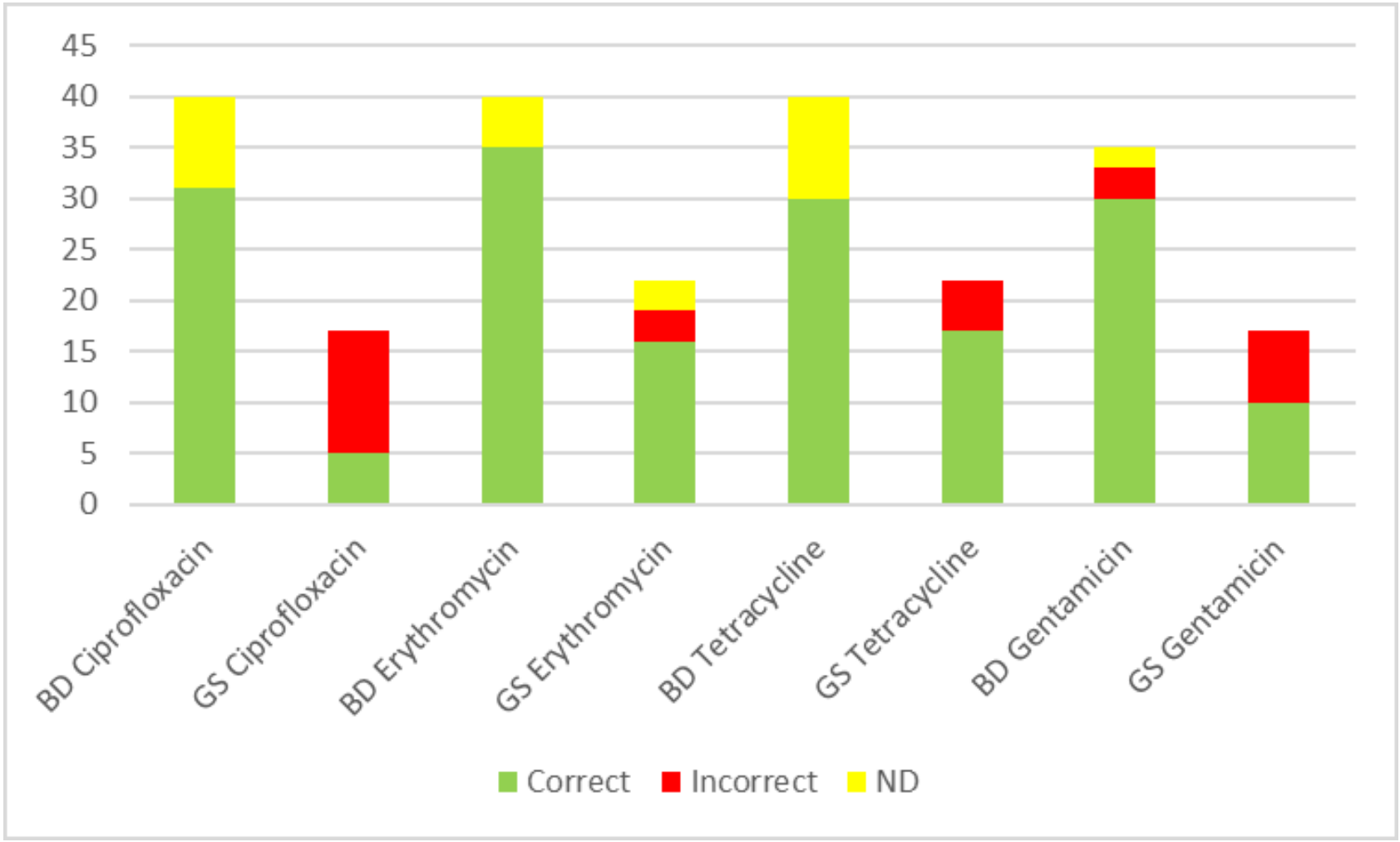


Campylobacter quantitative DD results (285 – all antimicrobials by laboratory



Campylobacter quantative DD results (285) all antimicrobials by strain

Campylobacter quantitative MIC result (243) by antimicrobial and method



Overall no of correct: GS 64% BD: 98% (ex ND´s)

Campylobacter quantitative MIC results (233) all antimicrobials by laboratory

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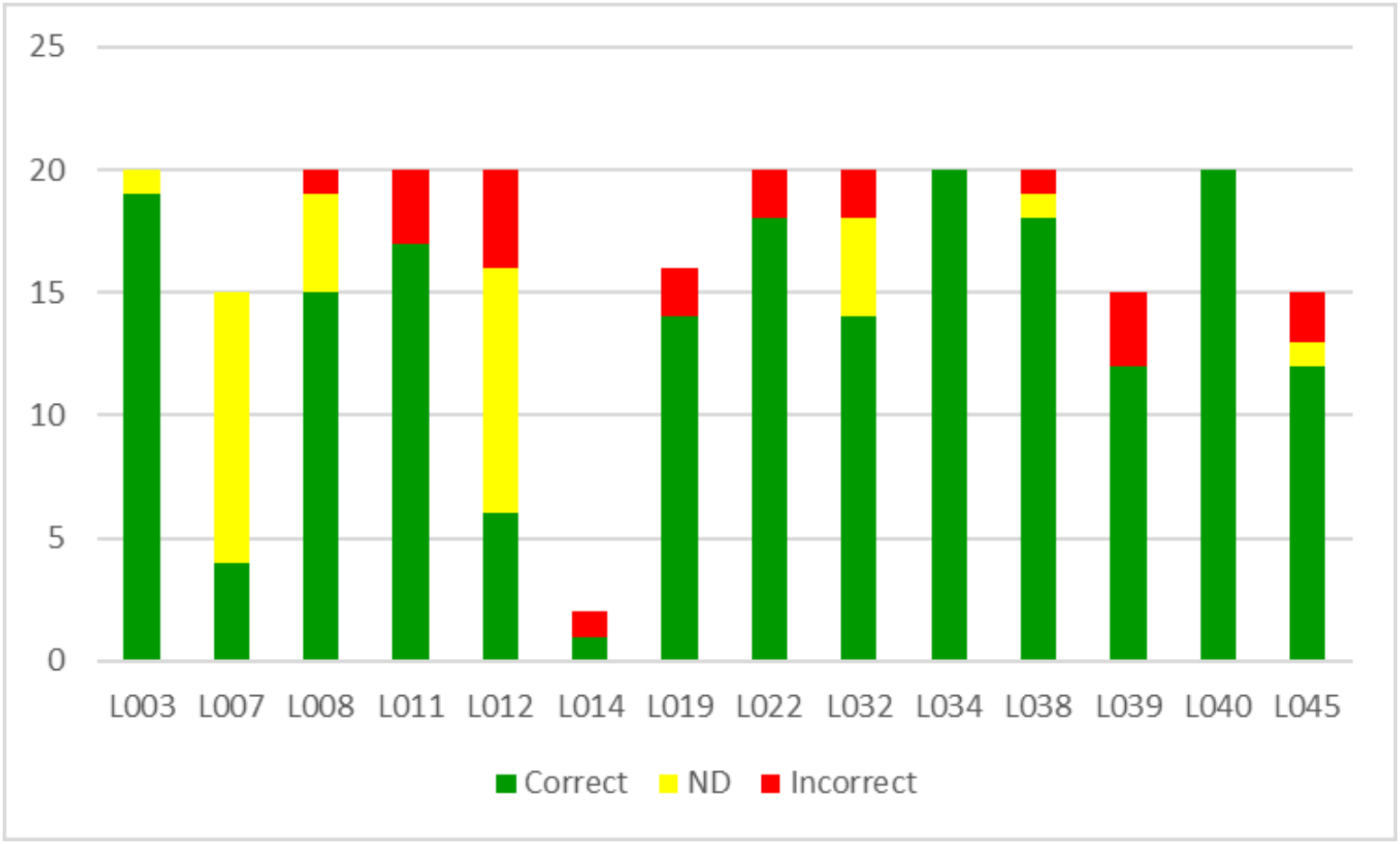

Overall no of correct: GS 64% BD: 98% (ex ND´s)

Campylobacter quantitative MIC results (233), all antimicrobials by strain

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CAMPYLOBACTER PREDICTED PHENOTYPES FROM WGS

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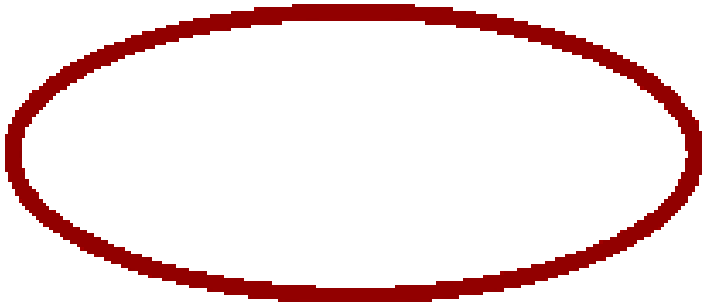
Predicted phenotypes from WGS data by antimicrobial					
Antimicrobial	Correct	Incorrect	Incorrect NWT	Incorrect WT	Total
Ciprofloxacin	38	3	3	0	41
Erythromycin	40	1	1	0	41
Tetracycline	39	2	2	0	41
Gentamicin	35	1	1	0	36
Total	152	7	7/100	0/52	159

Predicted phenotype from WGS data by antimicrobial			
Strain	Correct	Incorrect	Total
EQA_AST.C22.0001	35		35
EQA_AST.C22.0002	26	1	27
EQA_AST.C22.0003	27		27
EQA_AST.C22.0004	31	4	35
EQA_AST.C22.0005	33	2	35
Total	152	7	159

Predicted phenotype from WGS data by laboratory			
Lab no.	Correct	Incorrect	Total
L003	19	1	20
L011	20		20
L014	11	1	12
L017	11	1	12
L022	20		20
L032	20		20
L033	17	3	20
L044	15		15
L045	19	1	20
Total	152	7	159



Conclusions *Campylobacter*

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- EQA8 – highest participation ever recovered
- Overall correspondence between expected and reported results
- Results indicate that BD MIC methods are “better” than GS MIC methods
- Nine laboratories used WGS to predict resistance – with success !!
- No common laboratory problem identified
- **Results indicate that it is possible to compare phenotypic DD and MIC AST Campylobacter results from NPHRLs across Europe**

Acknowledgements

- All participating laboratories
- ECDC (Therese)
- EUCAST (Erika)
- EQA-team at SSI (Gosia)

IN CASE OF QUESTIONS

STATENS SERUM INSTITUT

- Contact SSI at any time = ast.eqa@ssi.dk
- Consult the EUCAST webpage (www.eucast.org)
- Consult the FWD AMR-RefLabCap (fwdamr@ssi.dk)

Thank you for your attention !