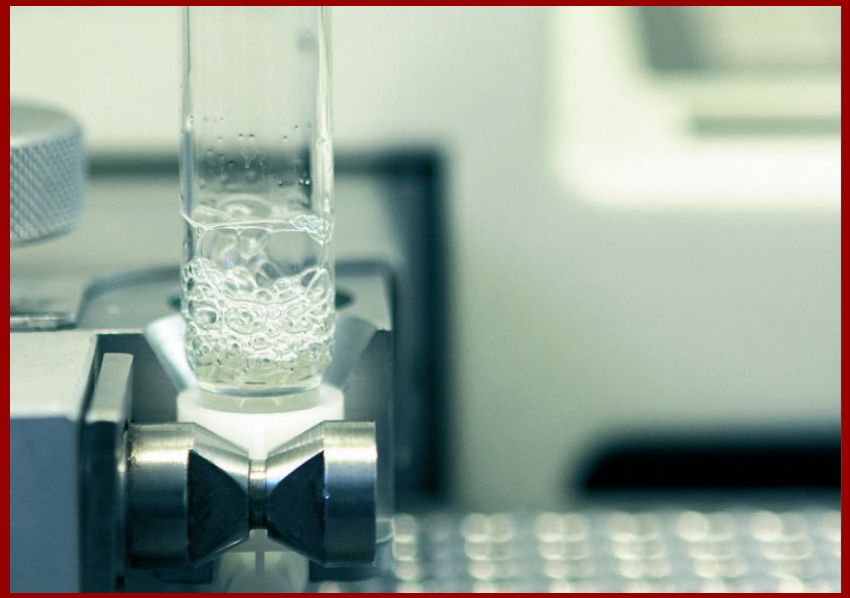
Update from

EURL-AR



Rene S. Hendriksen
Research group of Global Capacity Building
National Food Institute, Technical University of Denmark

17th EURL AR workshop, 23-24 May 2023

Activity: *To ensure availability and use of high quality methods and high quality performance by NRLs*

- Sub-activity 1.1 (Providing NRLs with details and guidance on laboratory methods)

- Manuscript on the ESBL/AmpC protocol has been submitted for publication to provide the supporting data – the background, how was it developed, the concerns and for what purpose

Activity: *To ensure availability and use of high quality methods and high quality performance by NRLs*

- Sub-activity 1.1 (Providing NRLs with details and guidance on laboratory methods)

- The EURL participated in a number of EFSA expert meetings facilitating the scientific report entitled "Technical specifications for a baseline survey on the prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) in pigs"

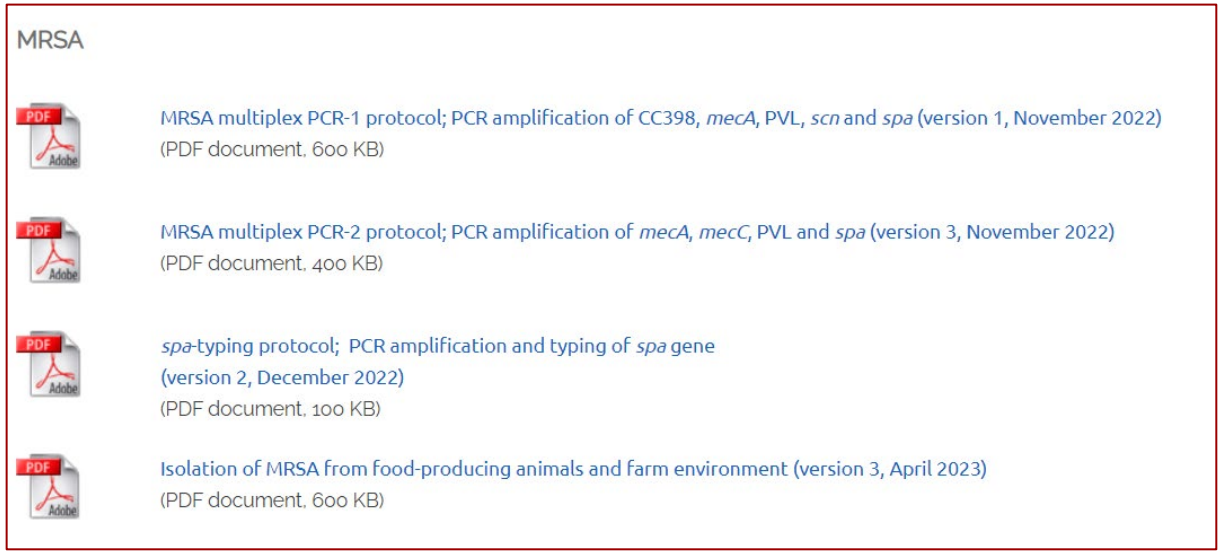
- And in drafting the associated legislation "Commission Implementing Decision of XXX - amending Commission Implementing Decision (EU) 2020/1729 as regards monitoring of methicillin-resistant *Staphylococcus aureus* (MRSA) in fattening pigs"

Activity: *To ensure availability and use of high quality methods and high quality performance by NRLs*

- Sub-activity 1.1 (Providing NRLs with details and guidance on laboratory methods)

- Developed four protocols made available from the EURL website prior to 2023 (though, was not part of the workplan for 2021-2022):
  - MRSA multiplex PCR-1 protocol; PCR amplification of CC398, *mecA*, PVL, *scn* and *spa*
  - MRSA multiplex PCR-2 protocol; PCR amplification of *mecA, mecC,* PVL and *spa*
  - spa-typing protocol; PCR amplification and typing of *spa* gene
  - Isolation of methicillin-resistant *Staphylococcus aureus* (MRSA) from food-producing animals and farm environment

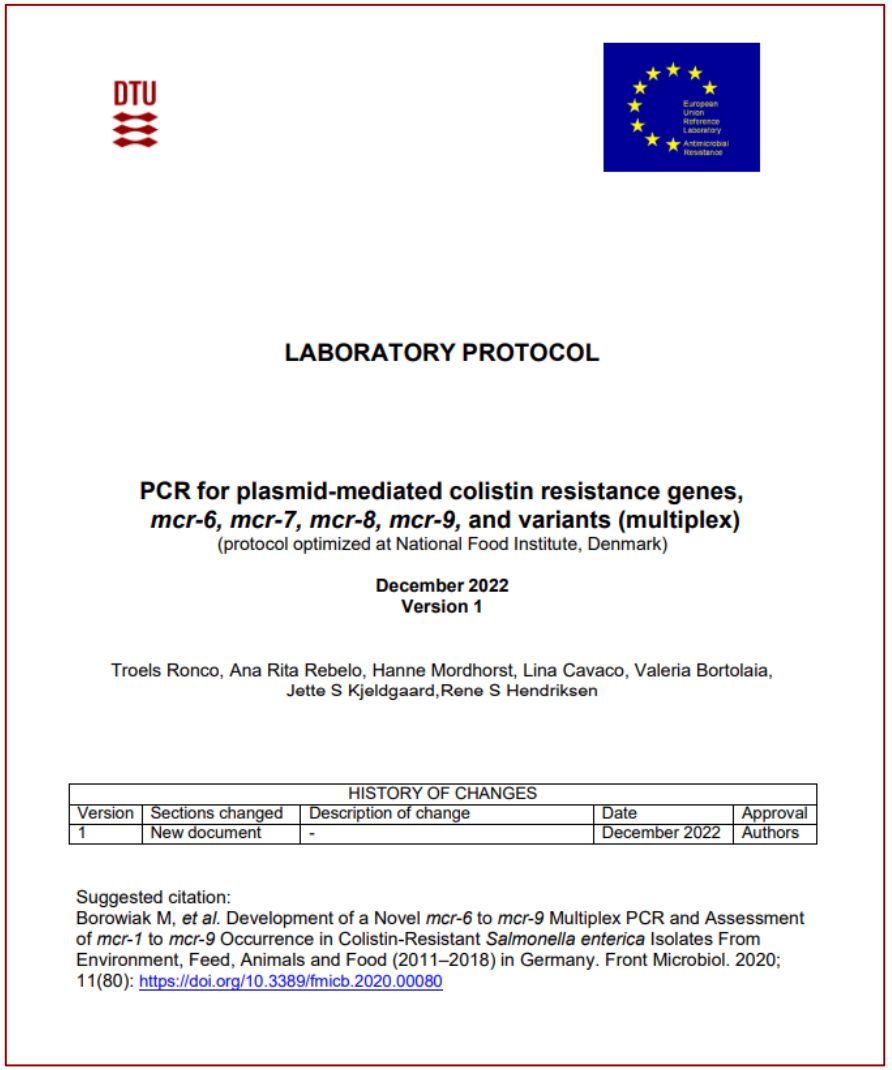
  (available on https://www.eurl-ar.eu/protocols.aspx)



Activity: *To ensure availability and use of high quality methods and high quality performance by NRLs*

- Sub-activity 1.1 (Providing NRLs with details and guidance on laboratory methods)

- Protocol of the extended multiplex PCR for detection of transferable colistin resistance genes mcr-1 to mcr-9 was developed in the reporting period and posted online (https://www.eurl-ar.eu/protocols.aspx)



LABORATORY PROTOCOL

PCR for plasmid-mediated colistin resistance genes, *mcr-6, mcr-7, mcr-8, mcr-9,* and variants (multiplex)
(protocol optimized at National Food Institute, Denmark)

December 2022
Version 1

Troels Ronco, Ana Rita Rebelo, Hanne Mordhorst, Lina Cavaco, Valeria Bortolaia, Jette S Kjeldgaard,Rene S Hendriksen

HISTORY OF CHANGES				
Version	Sections changed	Description of change	Date	Approval
1	New document	-	December 2022	Authors

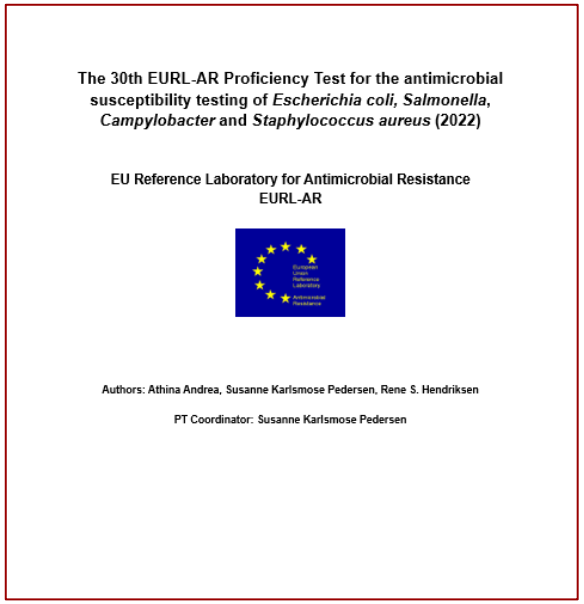
Suggested citation:
Borowiak M, *et al.* Development of a Novel *mcr-6* to *mcr-9* Multiplex PCR and Assessment of *mcr-1* to *mcr-9* Occurrence in Colistin-Resistant *Salmonella enterica* Isolates From Environment, Feed, Animals and Food (2011–2018) in Germany. Front Microbiol. 2020; 11(80): https://doi.org/10.3389/fmicb.2020.00080

Activity: *To ensure availability and use of high quality methods and high quality performance by NRLs*

- Sub-activity 1.1 (Coordination and organization of inter-laboratory comparative testing)

- Trial 1 – Phenotypic AST
  - *Salmonella*
  - *Campylobacter*
  - *Escherichia coli*
  - *Staphylococcus aureus*

  Test material sent in October 2022, data analysis and report in review


The 30th EURL-AR Proficiency Test for the antimicrobial susceptibility testing of *Escherichia coli*, *Salmonella*, *Campylobacter* and *Staphylococcus aureus* (2022)

EU Reference Laboratory for Antimicrobial Resistance
EURL-AR

Authors: Athina Andrea, Susanne Karlsmose Pedersen, Rene S. Hendriksen

PT Coordinator: Susanne Karlsmose Pedersen

- Trial 2 – Matrix EQA
  - Qualitative detection of ESBL and AmpC producing *E. coli* from a matrix of caecal and food samples (chicken/chicken meat)

  Test material sent in November 2022, data analysis and report being finalized

Activity: *To ensure availability and use of high quality methods and high quality performance by NRLs*

- Sub-activity 1.1 (Coordination and organization of inter-laboratory comparative testing)

- Trial 3 – DTU Genomic PT (October 2022)
  - Assessment of DNA extraction, purification, library-preparation, and WGS of six bacterial cultures of *S. aureus, E. coli* and *E. faecium/E. faecalis* strains
  - Results to participants released
    - on sequence quality in participants' submitted FASTQ files
    - on bioinformatic analysis to detect antimicrobial resistance (AMR) genes and mutations, and MLST

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  - Results to participants released
    - on sequence quality in participants' submitted FASTQ files
    - on bioinformatic analysis to detect antimicrobial resistance (AMR) genes and mutations, and MLST
  - Publications in the pipeline:
    - Sequence QC data from 2020 => a full paper (*in review*)
    - Sequence QC data from 2021 and 2022 => a letter
    - Participants' bioinformatic analysis from 2021 and 2022 => a full paper
    - Participants' bioinformatic analysis from 2020 => letter

Scientific publications in preparation

Activity: *To ensure availability and use of high quality methods and high quality performance by NRLs*

- Sub-activity 1.1 (Coordination and organization of inter-laboratory comparative testing)

- Focus on allowing results evaluation and data analysis nearer to the results submission.

Activity: *To provide scientific and technical assistance to NRLs*

- Sub-activity 2.2 (Conducting training courses for NRLs)

- Online training course in using WGS for AMR surveillance (October 2022)
  - Pheno- and geno-typing exercises with Gram-positive bacteria (*Staphylococcus aureus* and *Enterococcus spp*.) with focus on genomic exercises to analyse for genes relevant for the MRSA screening
  - MIC reading exercise on *S. aureus*, focusing on trailing endpoints and MRSA detection.

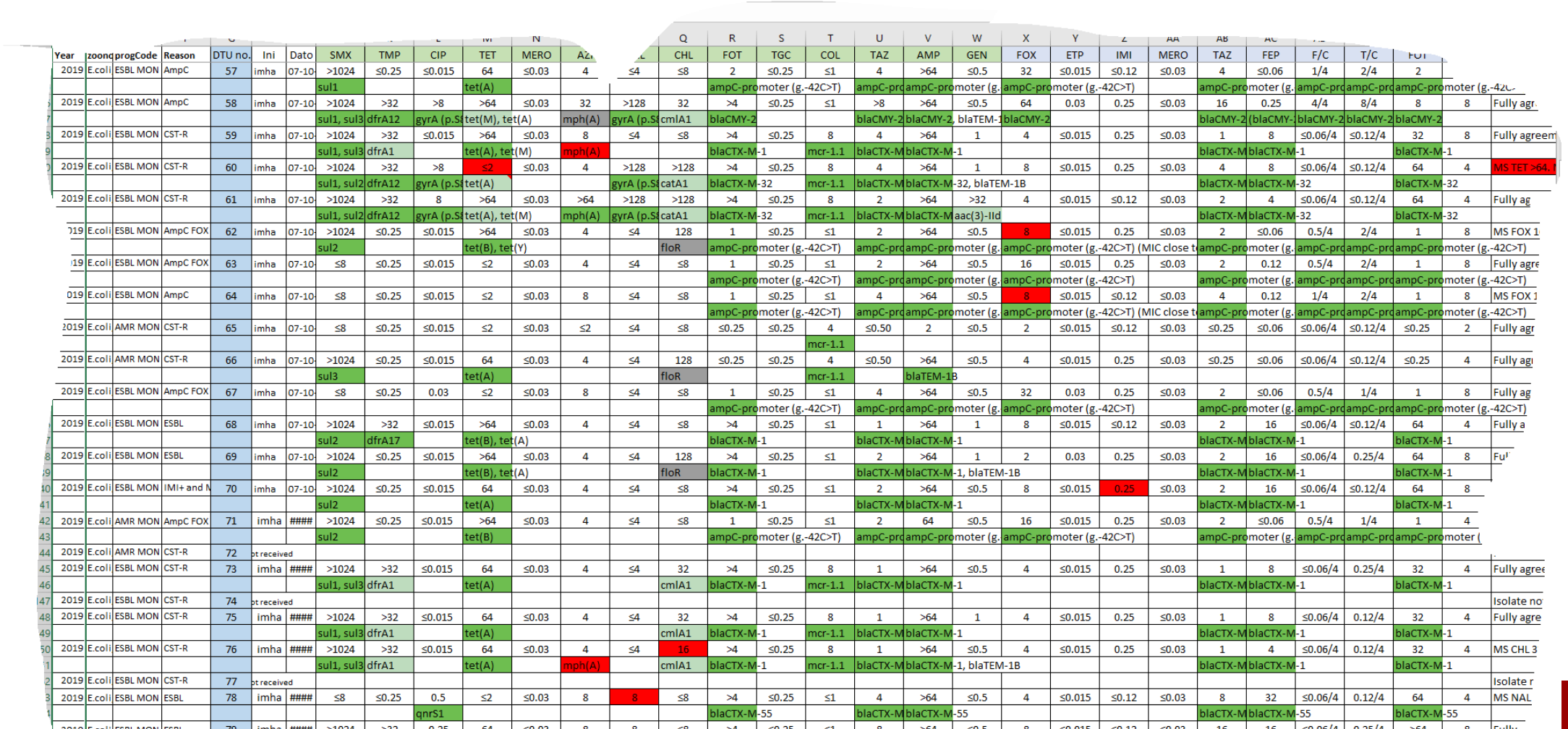
Activity: *To provide scientific and technical assistance to NRLs*

- Sub-activity 2.2 (Coordination and organization workshops among the NRLs)

- Held a joint workshop with FWD network in June 2022.

Activity: *To provide scientific and technical assistance to NRLs*

- Sub-activity 2.2 (Confirmatory testing)



Year	zoonq	progCode	Reason	DTU no.	Ini	Dato	SMX	TMP	CIP	TET	MERO	AZ		CHL	FOT	TGC	COL	TAZ	AMP	GEN	FOX	ETP	IMI	MERO	TAZ	FEP	F/C	T/C	FOT		
2019	E.coli	ESBL MON	AmpC	57	imha	07-10-	>1024	≤0.25	≤0.015	64	≤0.03	4	≤4	≤8	2	≤0.25	≤1	4	>64	≤0.5	32	≤0.015	≤0.12	≤0.03	4	≤0.06	1/4	2/4	2		
							sul1			tet(A)					ampC-promoter (g.-42C>T)			ampC-pro	ampC-promoter (g.		ampC-promoter (g.-42C>T)				ampC-promoter (g.	ampC-pro	ampC-pro	ampC-pro	ampC-promoter (g.-42C-		
2019	E.coli	ESBL MON	AmpC	58	imha	07-10-	>1024	>32	>8	>64	≤0.03	32	>128	32	>4	≤0.25	≤1	>8	>64	≤0.5	64	0.03	0.25	≤0.03	16	0.25	4/4	8/4	8	8	Fully agr
							sul1, sul3	dfrA12	gyrA (p.S	tet(M), tet(A)		mph(A)	gyrA (p.S	cmlA1	blaCMY-2			blaCMY-2	blaCMY-2, blaTEM-1	blaCMY-2					blaCMY-2	(blaCMY-2	blaCMY-2	blaCMY-2	blaCMY-2		
2019	E.coli	ESBL MON	CST-R	59	imha	07-10-	>1024	>32	≤0.015	>64	≤0.03	8	≤4	≤8	>4	≤0.25	8	4	>64	1	4	≤0.015	0.25	≤0.03	1	8	≤0.06/4	≤0.12/4	32	8	Fully agreem
							sul1, sul3	dfrA1		tet(A), tet(M)		mph(A)			blaCTX-M-1		mcr-1.1	blaCTX-M	blaCTX-M-1						blaCTX-M	blaCTX-M-1			blaCTX-M-1		
2019	E.coli	ESBL MON	CST-R	60	imha	07-10-	>1024	>32	>8	≤2	≤0.03	4	>128	>128	>4	≤0.25	8	4	>64	1	8	≤0.015	0.25	≤0.03	4	8	≤0.06/4	≤0.12/4	64	4	MS TET >64.
							sul1, sul2	dfrA12	gyrA (p.S	tet(A)			gyrA (p.S	catA1	blaCTX-M-32		mcr-1.1	blaCTX-M	blaCTX-M-32, blaTEM-1B						blaCTX-M	blaCTX-M-32			blaCTX-M-32		
2019	E.coli	ESBL MON	CST-R	61	imha	07-10-	>1024	>32	8	>64	≤0.03	>64	>128	>128	>4	≤0.25	8	2	>64	>32	4	≤0.015	≤0.12	≤0.03	2	4	≤0.06/4	≤0.12/4	64	4	Fully ag
							sul1, sul2	dfrA12	gyrA (p.S	tet(A), tet(M)		mph(A)	gyrA (p.S	catA1	blaCTX-M-32		mcr-1.1	blaCTX-M	blaCTX-M	aac(3)-IId					blaCTX-M	blaCTX-M-32			blaCTX-M-32		
019	E.coli	ESBL MON	AmpC FOX	62	imha	07-10-	>1024	≤0.25	≤0.015	>64	≤0.03	4	≤4	128	1	≤0.25	≤1	2	>64	≤0.5	8	≤0.015	0.25	≤0.03	2	≤0.06	0.5/4	2/4	1	8	MS FOX 1
							sul2			tet(B), tet(Y)				floR	ampC-promoter (g.-42C>T)			ampC-pro	ampC-promoter (g.		ampC-promoter (g.-42C>T) (MIC close t				ampC-promoter (g.	ampC-pro	ampC-pro	ampC-promoter (g.-42C>T)		
19	E.coli	ESBL MON	AmpC FOX	63	imha	07-10-	≤8	≤0.25	≤0.015	≤2	≤0.03	4	≤4	≤8	1	≤0.25	≤1	2	>64	≤0.5	16	≤0.015	0.25	≤0.03	2	0.12	0.5/4	2/4	1	8	Fully agre
															ampC-promoter (g.-42C>T)			ampC-pro	ampC-promoter (g.		ampC-promoter (g.-42C>T)				ampC-promoter (g.	ampC-pro	ampC-pro	ampC-promoter (g.-42C>T)		
019	E.coli	ESBL MON	AmpC	64	imha	07-10-	≤8	≤0.25	≤0.015	≤2	≤0.03	8	≤4	≤8	1	≤0.25	≤1	4	>64	≤0.5	8	≤0.015	≤0.12	≤0.03	4	0.12	1/4	2/4	1	8	MS FOX 1
															ampC-promoter (g.-42C>T)			ampC-pro	ampC-promoter (g.		ampC-promoter (g.-42C>T) (MIC close t				ampC-promoter (g.	ampC-pro	ampC-pro	ampC-promoter (g.-42C>T)		
2019	E.coli	AMR MON	CST-R	65	imha	07-10-	≤8	≤0.25	≤0.015	≤2	≤0.03	≤2	≤4	≤8	≤0.25	≤0.25	4	≤0.50	2	≤0.5	2	≤0.015	≤0.12	≤0.03	≤0.25	≤0.06	≤0.06/4	≤0.12/4	≤0.25	2	Fully agr
																	mcr-1.1													
2019	E.coli	AMR MON	CST-R	66	imha	07-10-	>1024	≤0.25	≤0.015	64	≤0.03	4	≤4	128	≤0.25	≤0.25	4	≤0.50	>64	≤0.5	4	≤0.015	0.25	≤0.03	≤0.25	≤0.06	≤0.06/4	≤0.12/4	≤0.25	4	Fully agı
							sul3			tet(A)				floR			mcr-1.1		blaTEM-1B											
2019	E.coli	ESBL MON	AmpC FOX	67	imha	07-10-	≤8	≤0.25	0.03	≤2	≤0.03	8	≤4	≤8	1	≤0.25	≤1	4	>64	≤0.5	32	0.03	0.25	≤0.03	2	≤0.06	0.5/4	1/4	1	8	Fully ag
															ampC-promoter (g.-42C>T)			ampC-pro	ampC-promoter (g.		ampC-promoter (g.-42C>T)				ampC-promoter (g.	ampC-pro	ampC-pro	ampC-promoter (g.-42C>T)		
2019	E.coli	ESBL MON	ESBL	68	imha	07-10-	>1024	>32	≤0.015	>64	≤0.03	4	≤4	≤8	>4	≤0.25	≤1	1	>64	1	8	≤0.015	≤0.12	≤0.03	2	16	≤0.06/4	≤0.12/4	64	4	Fully a
							sul2	dfrA17		tet(B), tet(A)					blaCTX-M-1			blaCTX-M	blaCTX-M-1						blaCTX-M	blaCTX-M-1			blaCTX-M-1		
2019	E.coli	ESBL MON	ESBL	69	imha	07-10-	>1024	≤0.25	≤0.015	>64	≤0.03	4	≤4	128	>4	≤0.25	≤1	2	>64	1	2	0.03	0.25	≤0.03	2	16	≤0.06/4	0.25/4	64	8	Fu
							sul2			tet(B), tet(A)				floR	blaCTX-M-1			blaCTX-M	blaCTX-M-1, blaTEM-1B						blaCTX-M	blaCTX-M-1			blaCTX-M-1		
2019	E.coli	ESBL MON	IMI+ and N	70	imha	07-10-	>1024	≤0.25	≤0.015	64	≤0.03	4	≤4	≤8	>4	≤0.25	≤1	2	>64	≤0.5	8	≤0.015	0.25	≤0.03	2	16	≤0.06/4	≤0.12/4	64	8	
							sul2			tet(A)					blaCTX-M-1			blaCTX-M	blaCTX-M-1						blaCTX-M	blaCTX-M-1			blaCTX-M-1		
2019	E.coli	AMR MON	AmpC FOX	71	imha	####	>1024	≤0.25	≤0.015	>64	≤0.03	4	≤4	≤8	1	≤0.25	≤1	2	64	≤0.5	16	≤0.015	0.25	≤0.03	2	≤0.06	0.5/4	1/4	1	4	
							sul2			tet(B)					ampC-promoter (g.-42C>T)			ampC-pro	ampC-promoter (g.		ampC-promoter (g.-42C>T)				ampC-promoter (g.	ampC-pro	ampC-pro	ampC-promoter (		
2019	E.coli	AMR MON	CST-R	72	ot received																										
2019	E.coli	ESBL MON	CST-R	73	imha	####	>1024	>32	≤0.015	64	≤0.03	4	≤4	32	>4	≤0.25	8	1	>64	≤0.5	4	≤0.015	0.25	≤0.03	1	8	≤0.06/4	0.25/4	32	4	Fully agree
							sul1, sul3	dfrA1		tet(A)				cmlA1	blaCTX-M-1		mcr-1.1	blaCTX-M	blaCTX-M-1						blaCTX-M	blaCTX-M-1			blaCTX-M-1		
2019	E.coli	ESBL MON	CST-R	74	ot received																										Isolate no
2019	E.coli	ESBL MON	CST-R	75	imha	####	>1024	>32	≤0.015	64	≤0.03	4	≤4	32	>4	≤0.25	8	1	>64	1	4	≤0.015	0.25	≤0.03	1	8	≤0.06/4	0.12/4	32	4	Fully agre
							sul1, sul3	dfrA1		tet(A)				cmlA1	blaCTX-M-1		mcr-1.1	blaCTX-M	blaCTX-M-1						blaCTX-M	blaCTX-M-1			blaCTX-M-1		
2019	E.coli	ESBL MON	CST-R	76	imha	####	>1024	>32	≤0.015	64	≤0.03	4	≤4	16	>4	≤0.25	8	1	>64	≤0.5	4	≤0.015	0.25	≤0.03	1	4	≤0.06/4	0.12/4	32	4	MS CHL 3
							sul1, sul3	dfrA1		tet(A)		mph(A)		cmlA1	blaCTX-M-1		mcr-1.1	blaCTX-M	blaCTX-M-1, blaTEM-1B						blaCTX-M	blaCTX-M-1			blaCTX-M-1		
2019	E.coli	ESBL MON	CST-R	77	ot received																										Isolate r
2019	E.coli	ESBL MON	ESBL	78	imha	####	≤8	≤0.25	0.5	≤2	≤0.03	8	8	≤8	>4	≤0.25	≤1	4	>64	≤0.5	4	≤0.015	≤0.12	≤0.03	8	32	≤0.06/4	0.12/4	64	4	MS NAL
									qnrS1						blaCTX-M-55			blaCTX-M	blaCTX-M-55						blaCTX-M	blaCTX-M-55			blaCTX-M-55		

Activity: *To provide scientific and technical assistance to the European Commission and other organisations*

- Sub-activity 3.2 (provide scientific and technical assistance to others than EU Commission)

- Continued the work to assist in promoting and implementing a common international standard for harmonization of AMR monitoring and support the capacity building in some countries for AMR monitoring (phenotypic and genotypic)
  - Mainly been centered around FAO (InFARM), WHO Global AMR Surveillance System (GLASS), UNEP, and Fleming Fund

Activity: *To provide scientific and technical assistance to NRLs*

- On 20 March 2023, the EURL-AR hosted a virtual meeting with the EURL-AR network, EFSA and ECDC, where observations from the Austrian Agency for Health and Food Safety (AGES) were presented and discussed:
  - AGES experienced high rates for ertapenem non-susceptible *Campylobacter* from food animals and the data showed possible different wild-type distributions between *C. jejuni* and *C. coli*, as well as in between different animal types of the same species.
  - Included in the discussion was also Dr. Philippe Lehours, University of Bordeaux, France (AMR expert) and subsequent to the virtual meeting, discussions also with European Committee on Antimicrobial Susceptibility Testing (EUCAST).

Activity: *To provide scientific and technical assistance to NRLs*

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Application submitted to EFSA (May 2023):

CarbaCamp Assessment of phenotypic carbapenem susceptibility and genomic epidemiology of *Campylobacter* from animal, food and human domains

Thanks for your attention

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