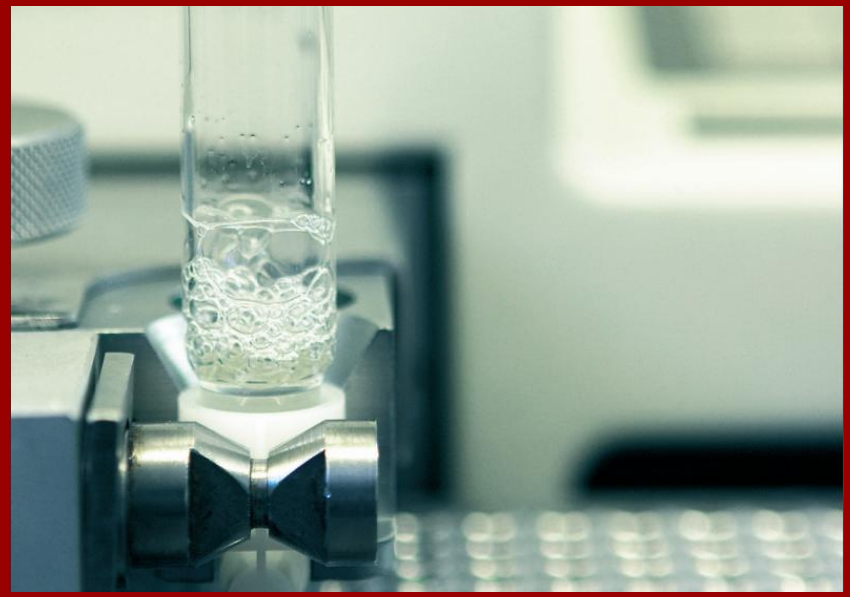
Update from EURL-AR

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



18th EURL-AR Workshop 2024
8th -9th October – Kgs. Lyngby, Denmark
Glassalen, Building 101

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

- Sub-activity 3.1.1.1 (Providing NRLs with details and guidance on laboratory methods)

- Updated in compliance with Commission Implementing Decision (EU) 2020/1729 of 17 November 2020 on the monitoring and reporting of antimicrobial resistance in zoonotic and commensal bacteria and repealing Implementing Decision 2013/652/EU)
  - References updated through-out the protocol stated that detection of carbapenemase producing E.coli including OXA-48 and OXA48-like producers is mandatory
    - Affecting background, contents, 1.6, 1.9, 3.1, 3.4, references, Appendix 2

LABORATORY PROTOCOL

Isolation of ESBL-, AmpC- and carbapenemase-producing *E. coli* from caecal samples

April 2024
Version 8

Version 8 reviewed and updated by: Rene Hendriksen

Authors of the document: Henrik Hasman, Yvonne Agersø, Rene Hendriksen, Lina M. Cavaco (DTU Food) and Beatriz Guerra-Roman (external expert)

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

- Sub-activity 3.1.1.3/ 4/ 5 (Providing NRLs with details and guidance on laboratory methods)

- The two MRSA PCR protocols were updated including the proper control ID strains
- MyDbFinder finalized for BioInformatic analysis
  - All should be ready for the baseline 2025 on MRSA

- Continue the updates of the protocol for short-read whole genome sequencing in addition to the development of a new WGS for long-read ONT technology
  - posted on the web pending

MRSA

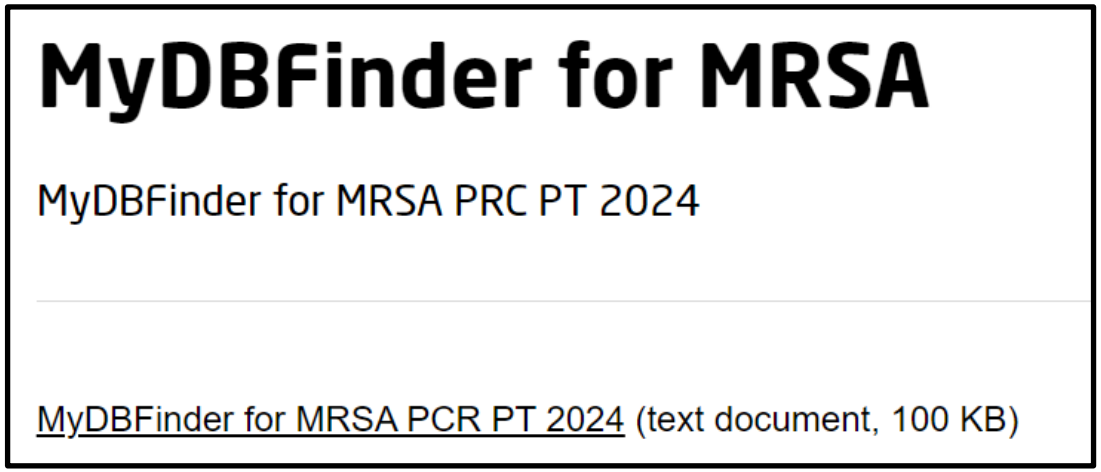
MRSA multiplex PCR-1 protocol; PCR amplification of CC398, mecA, PVL, scn and spa (version 1.1, April 2024) (PDF document, 600 KB)

MRSA multiplex PCR-2 protocol; PCR amplification of mecA, mecC, PVL and spa (version 3.1, April 2024) (PDF document, 400 KB)

Spa-typing protocol; PCR amplification and typing of spa gene (version 2, December 2022) (PDF document, 100 KB)

Isolation of MRSA from food-producing animals and farm environment (version 3, April 2023) (PDF document, 600 KB)

MLST typing (PDF document, 25 KB)



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

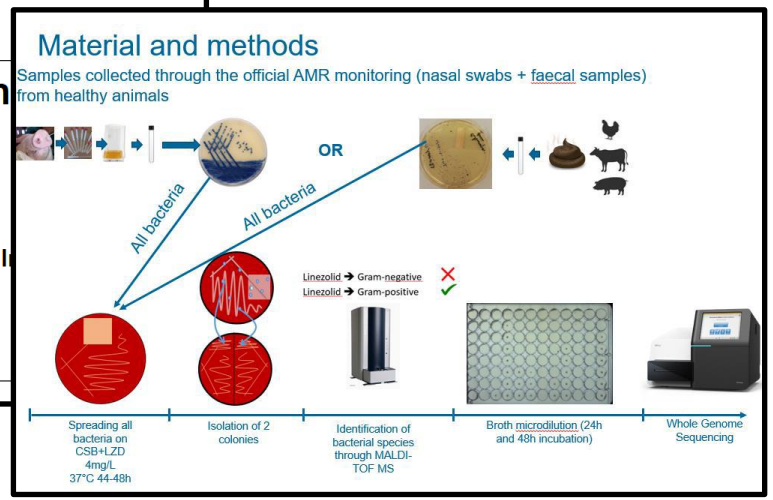
- Sub-activity 3.1.1.6 (Providing NRLs with details and guidance on laboratory methods)

- The EURL provided a “Freezed” version of the Resfinder database for the reporting period 2023 and 2024 including ESBL, AmpC and CP genes
  – will likely not be provided for 2025 and onwards due to lack updated ESBL, AmpC and CP genes, in ResFinder – thus the proposed discussion from EFSA

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

- Sub-activity 3.1.1.9 (Providing NRLs with details and guidance on laboratory methods)

- Initiate with the Veterinary Bacteriology Service, Sciensano, Belgium a voluntary linezolid resistance monitoring survey in order to assess the level of interest among the EU member states to participate in such monitoring activities



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

- Sub-activity 3.1.1.3 (Coordination and organization of inter-laboratory comparative testing)

- Trial 1 – Phenotypic AST (wrap up 2023 and launch of 2024)
  - *Salmonella*
  - *Campylobacter*
  - *Escherichia coli*

EQAS Reports

The 30th Proficiency Testing, 2022 – Escherichia coli, Salmonella, Campylobacter and Staphylococcus aureus (PDF document, 3.5 MB)

- Trial 2 – Matrix EQA (wrap up 2023 and launch of 2024)
  - Qualitative detection of ESBL and AmpC producing *E. coli* from a matrix of caecal and food samples (chicken/chicken meat)

- Trial 3 - MRSA PCR PT
  - Assessing ability to perform PCR according to PCR 1 and 2 multiplex

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

- Sub-activity 3.1.3.3 (Coordination and organization of inter-laboratory comparative testing)

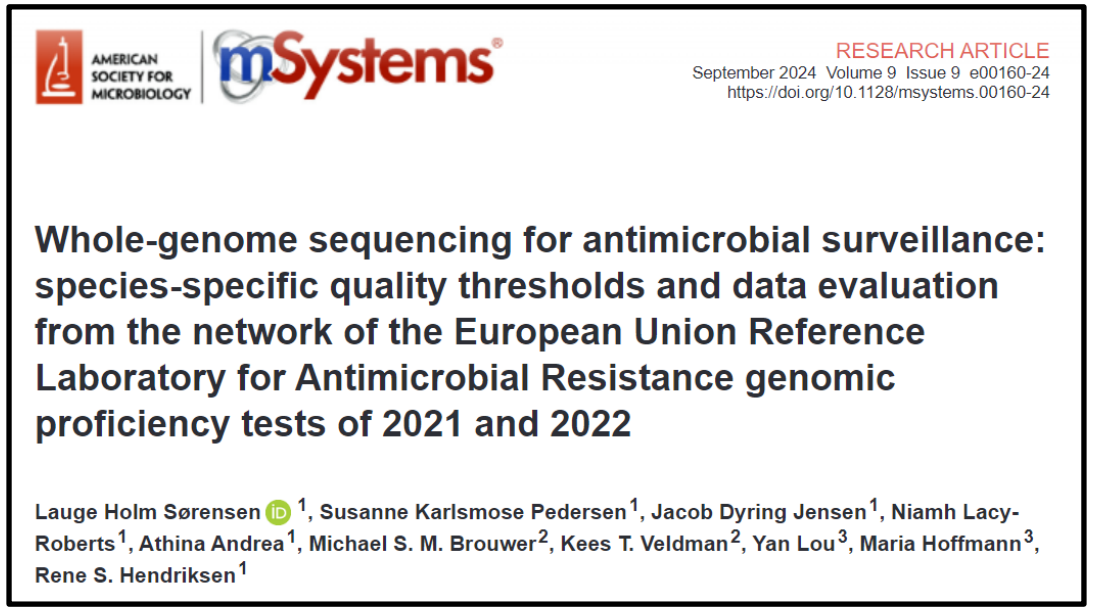
- Trial 4 – DTU Genomic PT
  - Assessment of library-preparation, and WGS of six bacterial cultures

  - 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

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

- Sub-activity 3.1.3.3 (Coordination and organization of inter-laboratory comparative testing)

- Trial 4 – DTU Genomic PT
  - Sequence QC data from 2021 and 2022 published



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RESEARCH ARTICLE
September 2024 Volume 9 Issue 9 e00160-24
https://doi.org/10.1128/msystems.00160-24

Whole-genome sequencing for antimicrobial surveillance: species-specific quality thresholds and data evaluation from the network of the European Union Reference Laboratory for Antimicrobial Resistance genomic proficiency tests of 2021 and 2022

Lauge Holm Sørensen [1], Susanne Karlsmose Pedersen [1], Jacob Dyring Jensen [1], Niamh Lacy-Roberts [1], Athina Andrea [1], Michael S. M. Brouwer [2], Kees T. Veldman [2], Yan Lou [3], Maria Hoffmann [3], Rene S. Hendriksen [1]

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

- Sub-activity 3.2.2 (Conducting training courses for NRLs)

National Food Institute

30 August 2024

Invitation to the EURL-AR Training Cour

On behalf of the EURL-AR team, I am pleased to invit
2024 at DTU Food in Kgs. Lyngby, Denmark.

The training course aims to ensure harmonization in
in zoonotic and commensal bacteria across the EU
tools to generate and use whole genome sequence (
ance with the EU Decision.

Course content

Members of the EURL-AR network have varying levels of expertise in WGS using Oxford Nanopore technologies and the course content will be broad to ensure that everyone gains new and relevant knowledge. The course is divided into three parts:

- 5 December 2024: Online session introducing Oxford Nanopore sequencing, one of the two most common sequencing technologies at present.
- 11-12 December 2024: Laboratory training for Oxford Nanopore sequencing (including high-molecular weight DNA extraction, library preparation, flow cell loading) at the Technical University of Denmark (DTU), Kgs. Lyngby, Denmark.
- 18 December 2024: Online session for bioinformatics analysis of Oxford Nanopore data.

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

- Sub-activity 3.2.4 (Coordination and organization workshops among the NRLs)

- Virtual workshop in 8th - 9th of Oct 2024

18th EURL-AR Workshop 2024

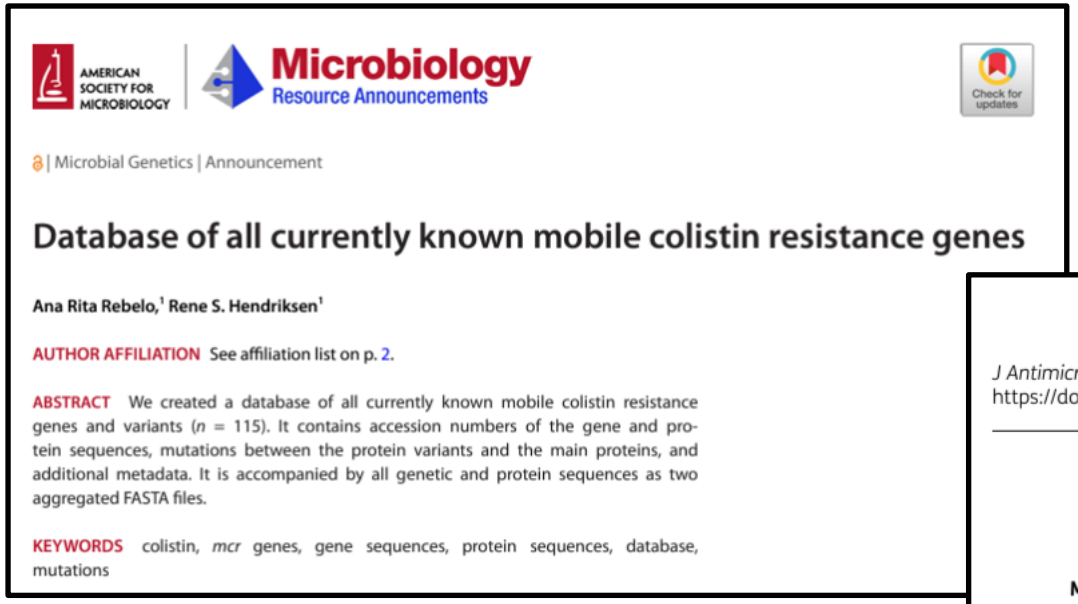
8th-9th October – Kgs. Lyngby, Denmark

Glassalen, Building 101

Tuesday, 8th October 2024		
9.00-9:20	**Welcome** **Meet and greet/ Introduction to the day's agenda**	Rene Hendriksen, EURL-AR
9:20-9:40	**Update from the EURL-AR**	Rene Hendriksen, EURL-AR
9:40-10:15	**Updates from EFSA on ongoing AMR activities**	Raquel García Fierro, EFSA
10:15-10:30	**Update from the EU Commission**	Martial Plantady, EU Commission /Virtual
10:30-11:00	Coffee break	
11:00-11:30	**EFSA/EURL-AR Confirmatory Testing**	Joana Mourão, EURL-AR
11:30-11:55	**2022 EUSR on AMR**	Raquel García Fierro, EFSA
11:55-12:20	**EFSA CarbaCamp- Assessment of phenotypic susceptibility, genomic epidemiology Campylobacter from animal, food and human**	Elisabeth Chang, EURL-AR

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

- Sub-activity 3.2.5 (Work Package) (Providing information on relevant research activities to NRLs)



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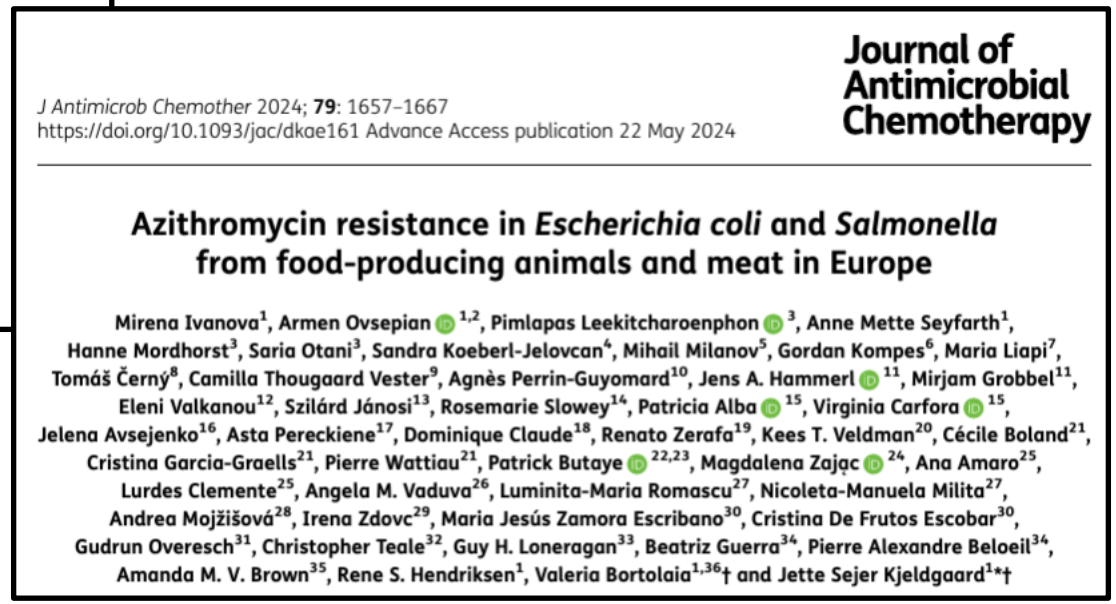
Database of all currently known mobile colistin resistance genes

Ana Rita Rebelo,[1] Rene S. Hendriksen[1]

AUTHOR AFFILIATION See affiliation list on p. 2.

ABSTRACT We created a database of all currently known mobile colistin resistance genes and variants (*n* = 115). It contains accession numbers of the gene and protein sequences, mutations between the protein variants and the main proteins, and additional metadata. It is accompanied by all genetic and protein sequences as two aggregated FASTA files.

KEYWORDS colistin, *mcr* genes, gene sequences, protein sequences, database, mutations



J Antimicrob Chemother 2024; **79**: 1657–1667
https://doi.org/10.1093/jac/dkae161 Advance Access publication 22 May 2024

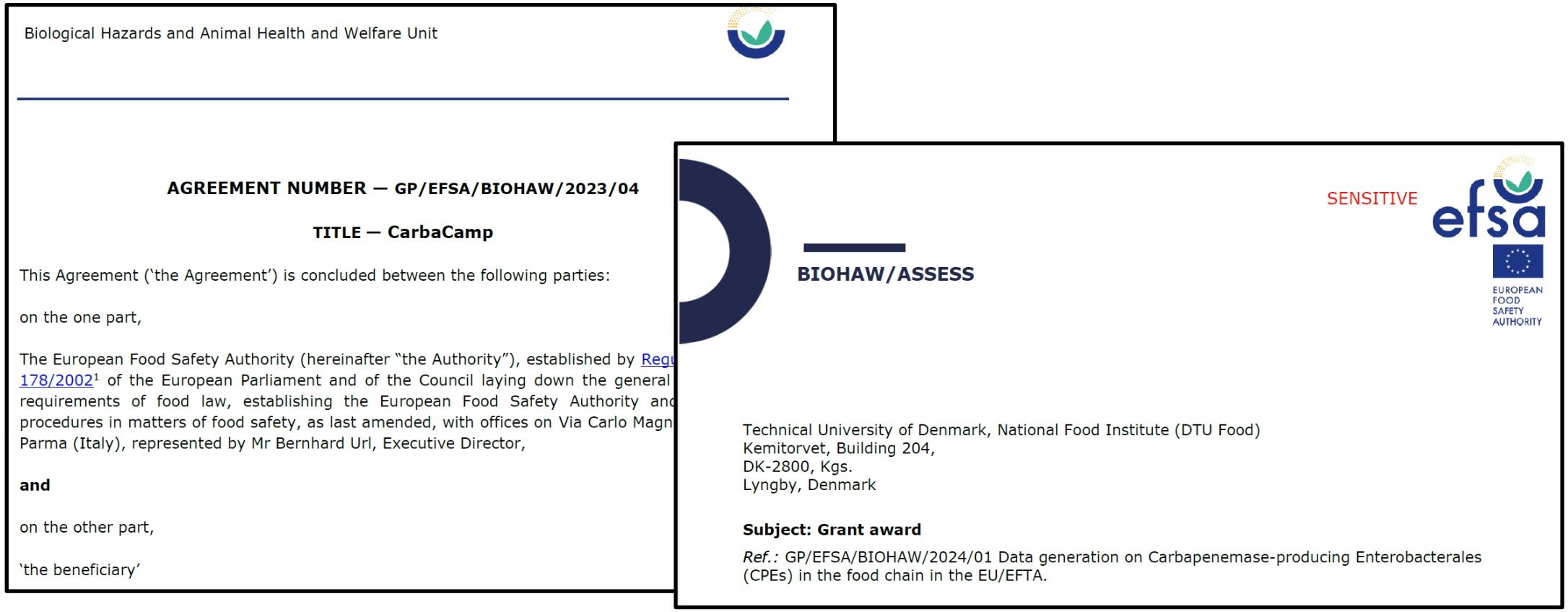
Journal of Antimicrobial Chemotherapy

Azithromycin resistance in *Escherichia coli* and *Salmonella* from food-producing animals and meat in Europe

Mirena Ivanova[1], Armen Ovsepian[1,2], Pimlapas Leekitcharoenphon[3], Anne Mette Seyfarth[1], Hanne Mordhorst[3], Saria Otani[3], Sandra Koeberl-Jelovcan[4], Mihail Milanov[5], Gordan Kompes[6], Maria Liapi[7], Tomáš Černý[8], Camilla Thougaard Vester[9], Agnès Perrin-Guyomard[10], Jens A. Hammerl[11], Mirjam Grobbel[11], Eleni Valkanou[12], Szilárd Jánosi[13], Rosemarie Slowey[14], Patricia Alba[15], Virginia Carfora[15], Jelena Avsejenko[16], Asta Pereckiene[17], Dominique Claude[18], Renato Zerafa[19], Kees T. Veldman[20], Cécile Boland[21], Cristina Garcia-Graells[21], Pierre Wattiau[21], Patrick Butaye[22,23], Magdalena Zając[24], Ana Amaro[25], Lurdes Clemente[25], Angela M. Vaduva[26], Luminita-Maria Romascu[27], Nicoleta-Manuela Milita[27], Andrea Mojžišová[28], Irena Zdovc[29], Maria Jesús Zamora Escribano[30], Cristina De Frutos Escobar[30], Gudrun Overesch[31], Christopher Teale[32], Guy H. Loneragan[33], Beatriz Guerra[34], Pierre Alexandre Beloeil[34], Amanda M. V. Brown[35], Rene S. Hendriksen[1], Valeria Bortolaia[1,36]† and Jette Sejer Kjeldgaard[1]*†

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

- Sub-activity 3.2.5 (Work Package) (Providing information on relevant research activities to NRLs)



Biological Hazards and Animal Health and Welfare Unit

AGREEMENT NUMBER — GP/EFSA/BIOHAW/2023/04

TITLE — CarbaCamp

This Agreement ('the Agreement') is concluded between the following parties:

on the one part,

The European Food Safety Authority (hereinafter "the Authority"), established by Regu
178/2002[1] of the European Parliament and of the Council laying down the general
requirements of food law, establishing the European Food Safety Authority and
procedures in matters of food safety, as last amended, with offices on Via Carlo Magn
Parma (Italy), represented by Mr Bernhard Url, Executive Director,

and

on the other part,

'the beneficiary'

SENSITIVE

BIOHAW/ASSESS

Technical University of Denmark, National Food Institute (DTU Food)
Kemitorvet, Building 204,
DK-2800, Kgs.
Lyngby, Denmark

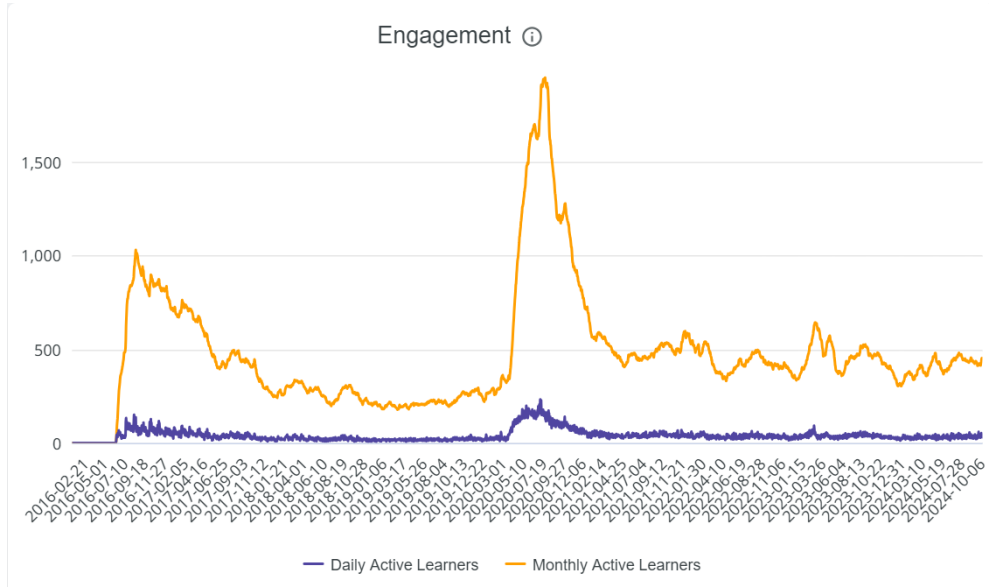
Subject: Grant award

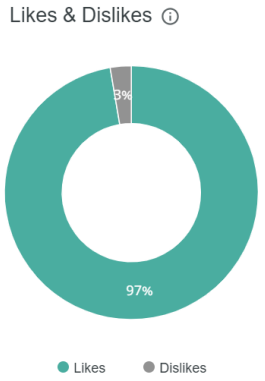
Ref.: GP/EFSA/BIOHAW/2024/01 Data generation on Carbapenemase-producing Enterobacterales (CPEs) in the food chain in the EU/EFTA.

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

- Sub-activity 3.2.6 (Providing E-learning)
- In 2023 and 2024, the EURL has re-recorded all old videos and include videos of the practical work in recognition that many videos are more than seven years old
  - A series of new E-learning lectures and video incl. hands on antimicrobial susceptibility testing and MALDI-TOF

263,258	66,706	41,180	14,632
Unique Visitors	Enrolled in Course	Started Course	Completed Course





2,347	4.7 / 5	4.7 / 5	495
Number of Star Ratings	Average Star Rating: All Courses	Average Star Rating: Your Course	Reviews

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

- Sub-activity 3.2.7 (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-	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-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-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-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	ESBL 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-pro	ampC-pro	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-pro	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 3.3.1 (Providing scientific and technical assistance to the EU Commission)

- Technical Specifications for a baseline survey on AMR in aquaculture animals

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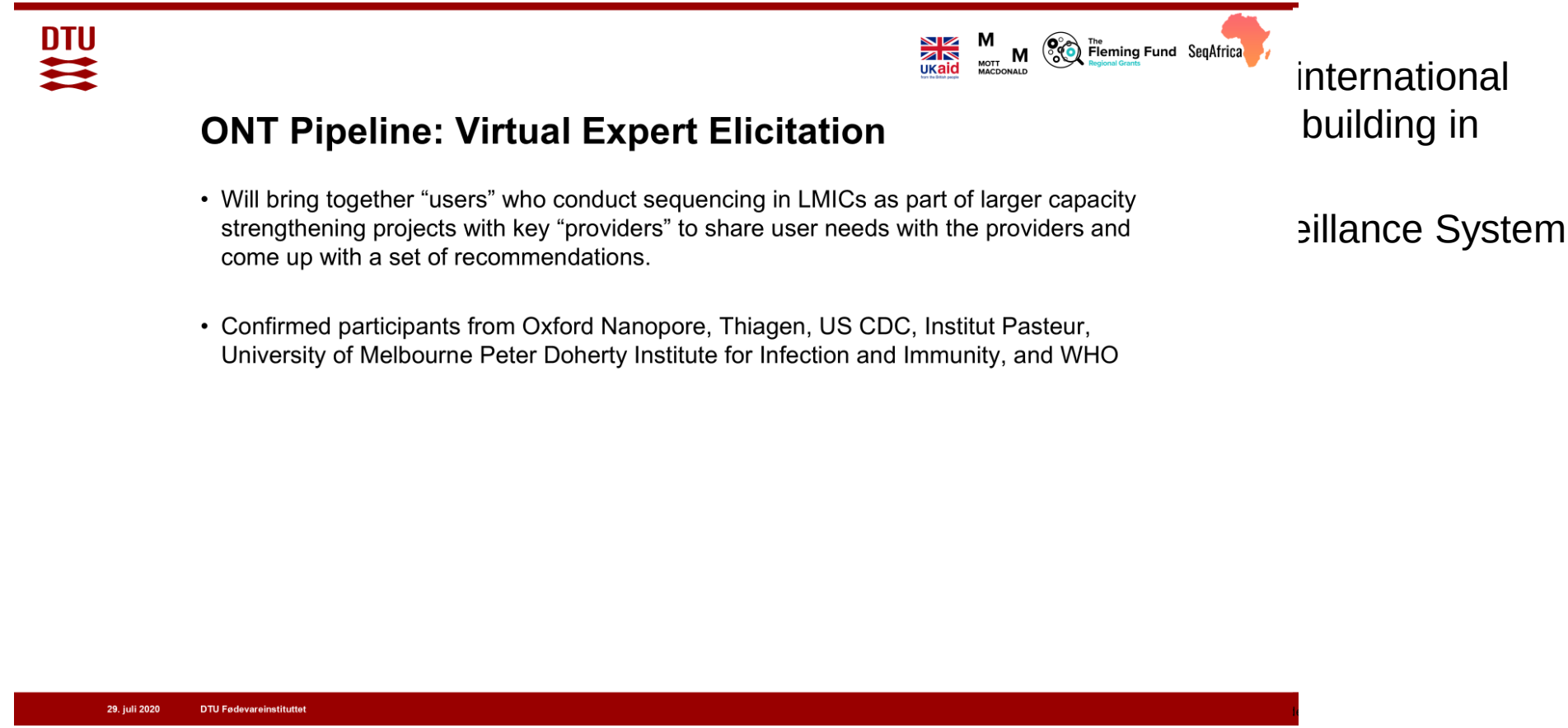
Technical specifications for a EU-wide baseline survey of antimicrobial resistance in bacteria from aquaculture animals

European Food Safety Authority (EFSA) | Marc Aerts | Sandrine Baron | Valeria Bortolaia | Rene Hendriksen | Beatriz Guerra | Anca Stoicescu | Pierre-Alexandre Beloeil

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

- Sub-activity 3.3.2 (provide scientific and technical assistance to others than EU Commission)



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Thank you for your attention

Prof. Rene S. Hendriksen, PhD

Head of Research Group Global Capacity Building

WHO Collaborating Centre for Antimicrobial Resistance in Food borne Pathogens and Genomics

European Union Reference Laboratory for Antimicrobial Resistance

FAO Reference Laboratory for Antimicrobial Resistance

National Food Institute, Technical University of Denmark

rshe@food.dtu.dk

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