

Confirmatory testing - summary of results (2021 data) and criteria for the upcoming testing (2022 data)

Mirena Ivanova

EURL-AR

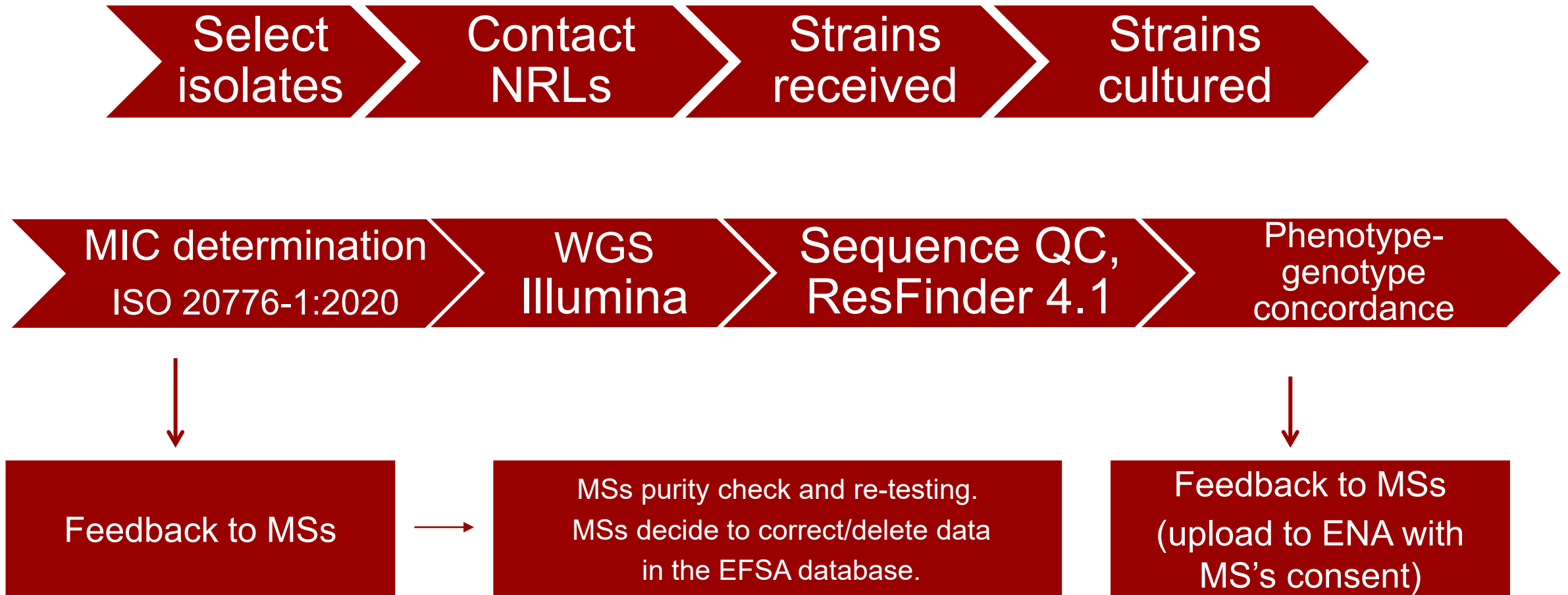
Term of Reference, EURL-AR

The EURL-AR will provide confirmatory testing on bacterial isolates of particular relevance upon request by the NRLs, the European Commission and EFSA:

- To support the activities related to the implementation of the Decision 2013/652/EU
- To confirm the AMR phenotypes observed by MSs
- To determine the AMR genotypes and to evaluate phenotype-genotype concordance

Relevant Material Transfer Agreements (MTAs) between the EURL and MSs ensure the legal aspects of strain ownership.

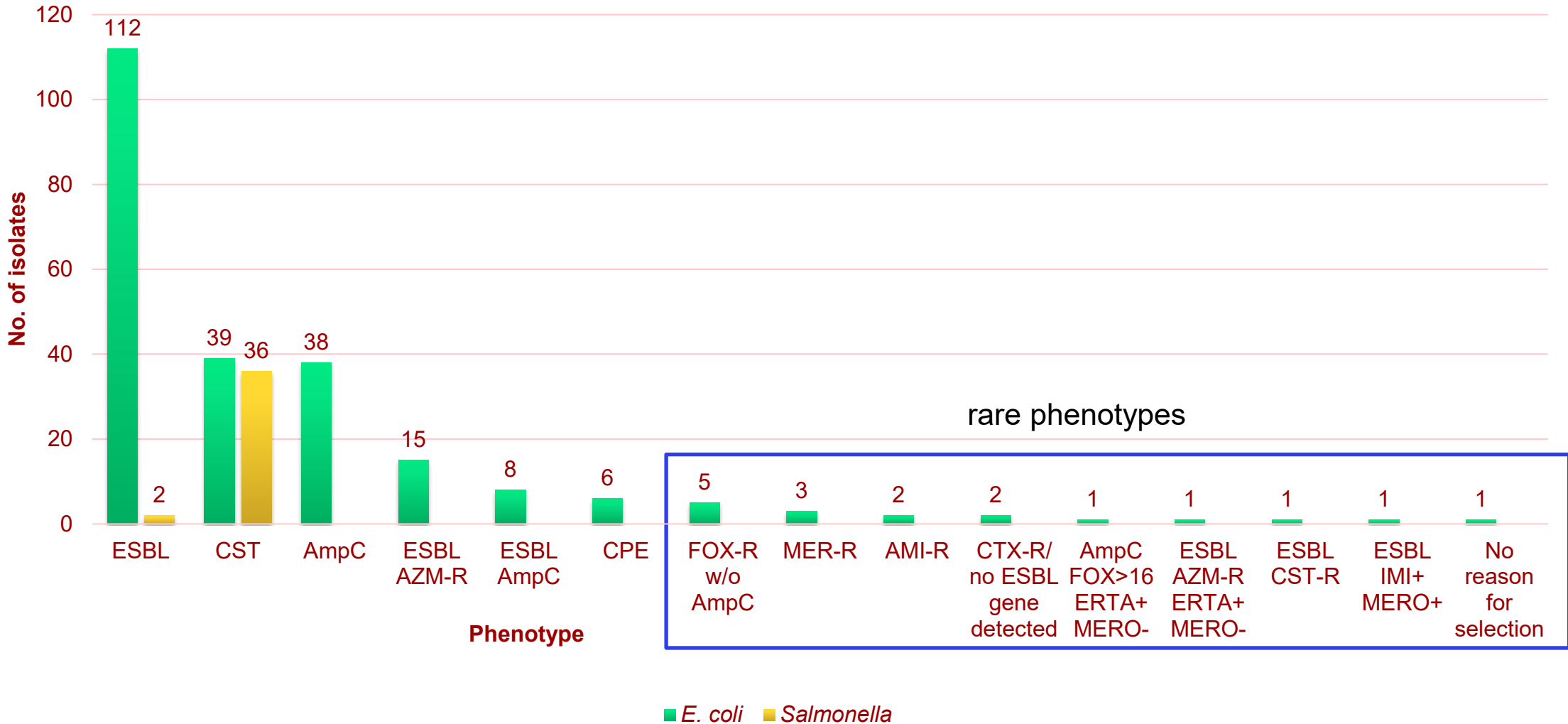
Workflow for the confirmatory testing activities



Reasons for selection of isolates by EFSA

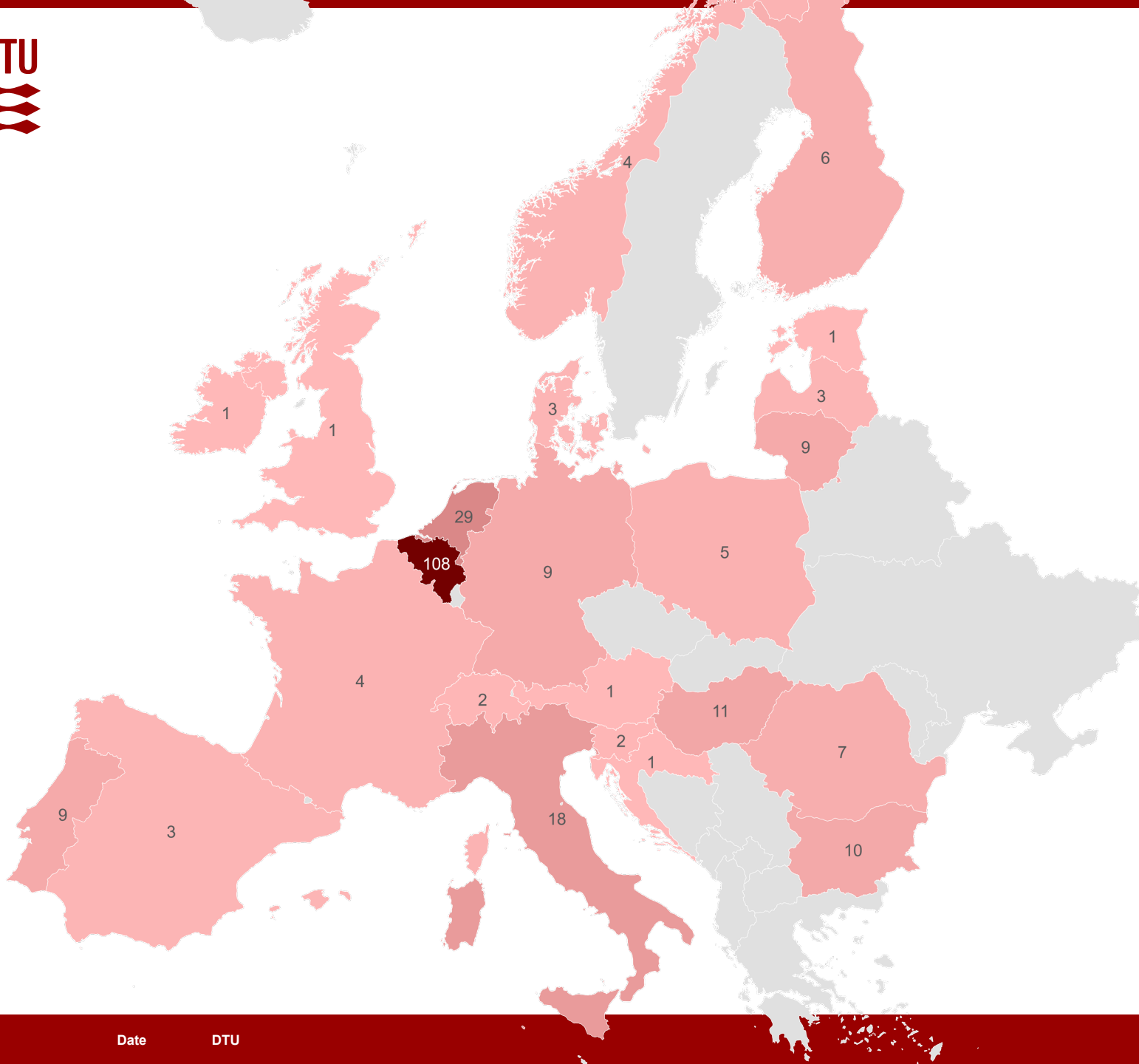
- The selection criteria for the 2021 isolates:
 - ✓ colistin resistance in *E. coli* and *Salmonella*
 - ✓ presumptive ESBL/AmpC profiles with or without azithromycin resistance
 - ✓ carbapenem resistance
 - ✓ Rare (other) phenotypes:
 - FOX-R w/o *ampC* promoter mutation
 - MER-R
 - AMI-R
 - CTX-R/ no ESBL gene detected
 - AmpC ERTA+ MERO-
 - ESBL AZM-R ERTA+ MERO-
 - ESBL CST-R
 - ESBL IMI+ MERO+
 - No reason for selection

Distribution of the isolates based on the selected phenotypes



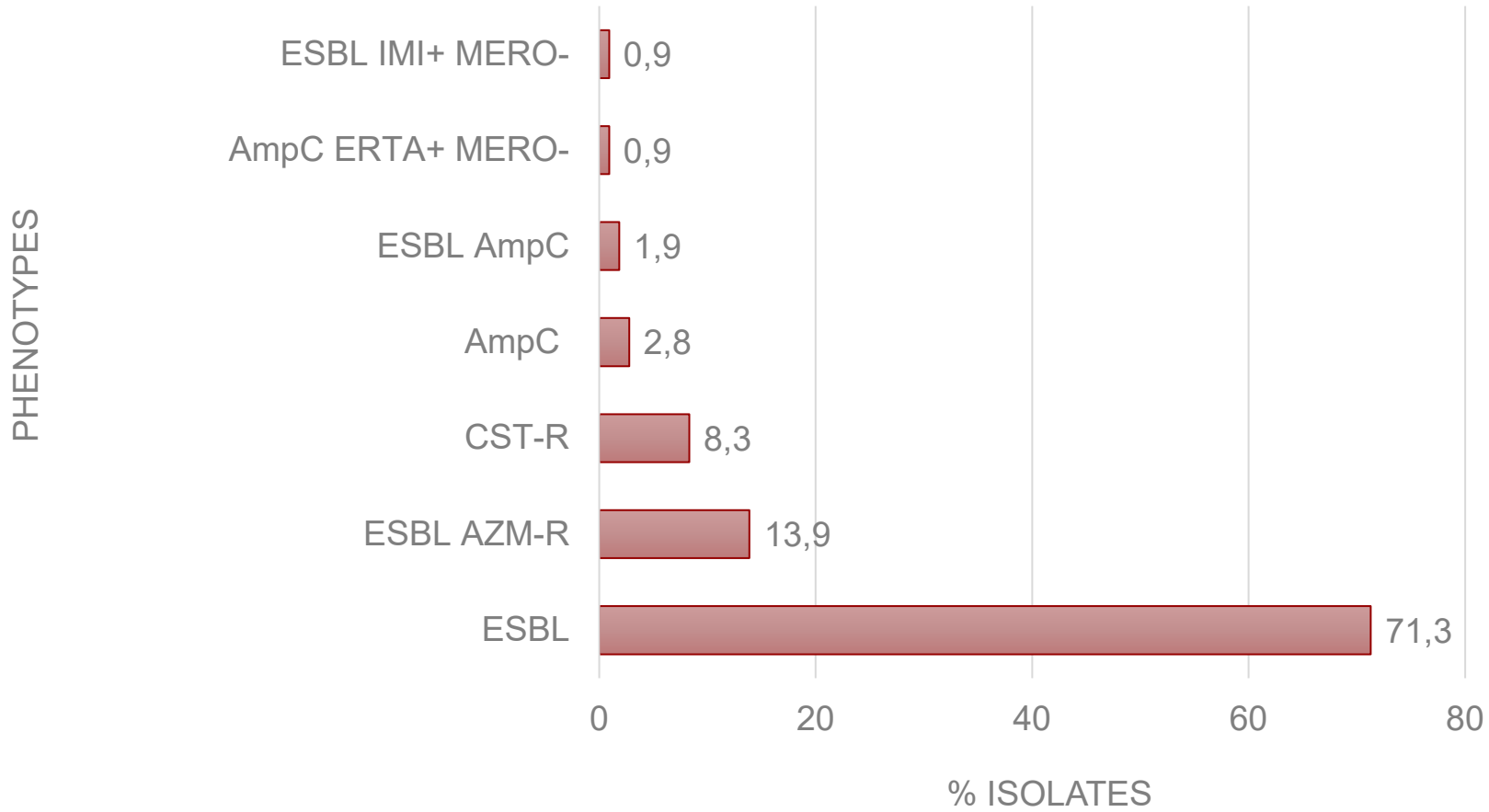
E. coli and *Salmonella* isolates in the 2021 confirmatory testing

- Of the 292 isolates selected by EFSA, 13 were not received by the EURL, 5 were contaminated, and 1 was *Citrobacter*.
- Final list of 273 isolates from 24 countries tested by the broth microdilution method.
- Of them, 235 were *E. coli* and 38 *Salmonella*.
- 36 of the 38 *Salmonella* were selected based on colistin resistance.



- Distribution of the 273 isolates by country of isolation.
- 108 (40%) isolates were chosen from Belgium – 107 *E. coli* and 1 *Salmonella*.

Phenotypes of Belgium isolates



Highlights from 2021 confirmatory testing

- 16 isolated had more than one two-fold dilution difference between EURL and MS, **which changed the susceptibility category** (re-test foreseen):
 - TET (1)
 - SMX (2)
 - NAL (1)
 - IMI (1)
 - FOX (1)
 - CST (3)
 - CHL (2)
 - AZI (5)
- 4 isolates have very different AMR profiles at the EURL compared to the submitted by the MS (this has been communicated to the country).

Highlights from 2021 confirmatory testing

- ***mef(C)-mph(G)***: 2 *E. coli* isolates carried the emerging tandem *mef(C)-mph(G)* genes previously shown to be located on plasmids and conferring high AZM resistance in *E. coli* and *Salmonella* (MIC \geq 64 mg/L).
- ***mph(A)***: In 6 AZM susceptible isolates, *mph(A)* was detected. It has also been observed in previous years.
- **AMP-S and FOX-R phenotype**: 2 *Salmonella* isolates (we observed in *E. coli* without any ESBL gene to be due to unfunctional OmpF porin).

Highlights from 2021 confirmatory testing

Resistant isolates with undescribed/unknown mechanisms of resistance:

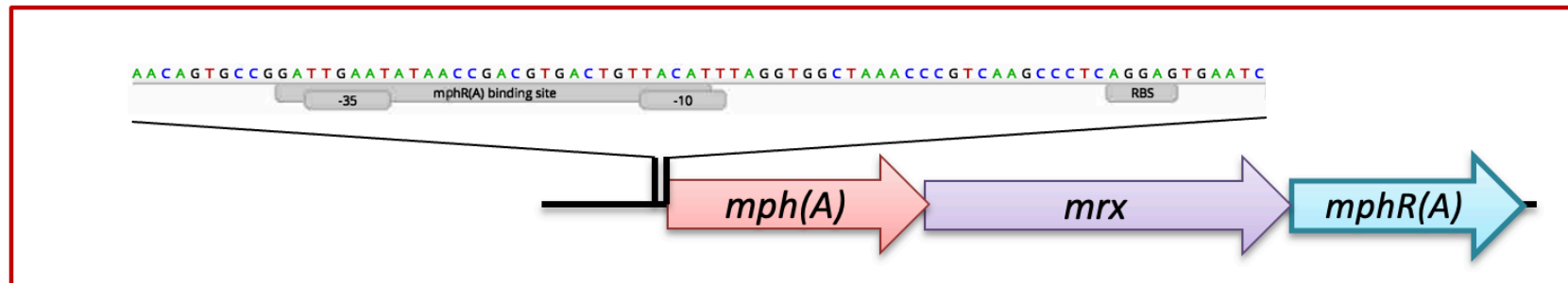
- **AZM:** 4 isolates. *Note:* no screening for point mutations in *rplV*, *rplD* or *23S rRNA* genes were performed (not included in PointFinder)
 - **COL:** 14 *Salmonella* isolates (common observation).
 - **SMX** (sulfamethoxazole): 2 susceptible isolates with *sul1* and *sul2*; and 1 resistant isolate without a gene
 - **GEN:** 1 susceptible isolate with *aac(3)-IV*
 - **CHL, NAL, CIP:** resistant isolate each without a gene or detection of a gene with a resistant phenotype.
- Some of these cases could also be caused by discrepancies in either the broth microdilution method or in the sequencing data.
- Detailed analyses are needed to elucidate the reason behind these observations.

AZM mechanism of resistance

- 1,033 *E. coli* and 286 *Salmonella* screened in 2014-2018 confirmatory testing data.
- 190 *mph(A)*-harbouring isolates detected.
- Two *mph(A)* operons structures, in concordance with the phenotypic data.

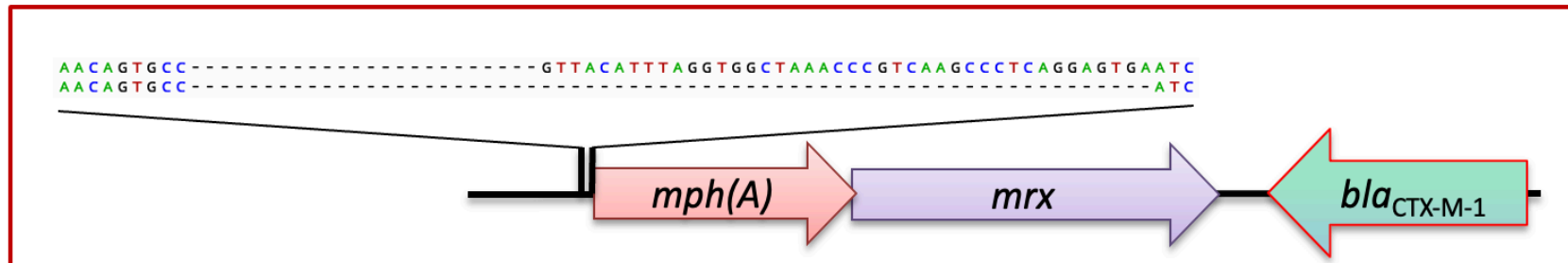
73% - MIC > 16 mg/L

Non-WT



27% - MIC ≤ 16 mg/L

WT



For submission to JAC

Azithromycin Resistance in *Escherichia coli* and *Salmonella* from Food and Food-Producing Animals in Europe

Mirena Ivanova^{1*}, Armen Ovsepien^{1*}, Pimlapas Leekitcharoenphon¹, Anne Mette Seyfarth¹, Hanne Mordhorst¹, Saria Otani¹, Sandra Koeberl-Jelovcan², Mihail Milanov³, Gordan Kompes⁴, Maria Liapi⁵, Tomáš Černý⁶, Camilla Thougard Vester⁷, Agnès Perrin-Guyomard⁸, Jens Hammerl⁹, Mirjam Grobbel⁹, Eleni Valkanou¹⁰, Szilárd Jánosi¹¹, Rosemarie Slowey¹², Patricia Alba¹³, Virginia Carfora¹³, Jelena Avsejenko¹⁴, Ambrozeviciene Ceslova¹⁵, Asta Pereckiene¹⁵, Snieguole Sceponaviciene¹⁵, Dominique Claude¹⁶, Renato Zerafa¹⁷, Kees Veldman¹⁸, Cécile Boland¹⁹, Cristina Garcia-Graells¹⁹, Patrick Butaye¹⁹, Pierre Wattiau¹⁹, Magdalena Zajac²⁰, Ana Amaro²¹, Lurdes Clemente²¹, Mihail Vaduva Angela²², Luminita-Maria Romascu²³, Nicoleta-Manuela Milita²³, Andrea Mojžišová²⁴, Irena Zdovc²⁵, Maria Jesús Zamora Escribano²⁶, Cristina De Frutos Escobar²⁶, Karl Pedersen²⁷, Gudrun Overesch²⁸, Christopher Teale²⁹, Amanda M.V. Brown³⁰, Guy H. Loneragan³⁰, Beatriz Guerra³¹, Pierre Alexandre Beloeil³¹, Rene S. Hendriksen¹, Jette Sejer Kjeldgaard¹, Valeria Bortolaia^{1,32}

Carbapenem resistance in 2021 confirmatory data

Country	Sample	MLST	Carbapenem resistance gene	Plasmid replicon gene	
Czechia	21 P BA 4003 4 K	898	blaNDM-5	IncY, IncR, IncX3 , IncX3, IncFIB(K), IncFIB	chromosome (IS30 and IS5)
Czechia	21 P BA 225 3 K	10	blaNDM-5	IncX3, IncR	
Hungary	M2021_10044802_2_E	405	blaNDM-5	p0111, IncFIB , IncX4	
Hungary	M2021_10043982_E	405	blaNDM-5	p0111, IncFIB , IncX4	rep_cluster_1195
Hungary	M2021_10044824_1_E	405	blaNDM-5	p0111, IncFIB , IncX4	
Italy	21112463I110X6	5229	blaOXA-181	IncFIB, IncX4, IncI1, IncFIA	
Italy	21094270C326X6	5229	blaOXA-181	IncX3, IncFIB, IncFIC	chromosome (IS10A)
Italy	21098725F502X6	617	blaNDM-5	IncFIA , IncFIB , IncX4, IncI-alpha	
Italy	21102457L202X6	38	blaOXA-48	-	

PlasmidFinder & Mob-suite: Tool for clustering and reconstruction of plasmids from draft assemblies.

IncX1 – associated with blaOXA-181 in Italian samples.

Other that could carry OXA-181 are IncX1 plasmids

Carbapenem resistance in 2021 confirmatory data

Number of SNPs differences in the 9 carbapenamse producing isolates analysed by the CSI phylogeny:

			21_P_BA_225 _3_K	21_P_BA_4003 _4_K	M2021_10044824 _1_E	M2021_100439 _82_E	M2021_10044802 _2_E	21098725F50 2X6	21102457L20 2X6	21094270C32 6X6	21112463I11 0X6
Czechia	blaNDM-5	21_P_BA_225_3_K	0	3437	2130	2130	2135	1064	2215	3427	3434
Czechia	blaNDM-5	21_P_BA_4003_4_K	3437	0	4910	4910	4915	4127	5051	3068	3073
Hungary	blaNDM-5	M2021_10044824_1_E	2130	4910	0	8	11	2242	2610	4856	4859
Hungary	blaNDM-5	M2021_10043982_E	2130	4910	8	0	13	2242	2610	4856	4859
Hungary	blaNDM-5	M2021_10044802_2_E	2135	4915	11	13	0	2247	2617	4861	4864
Italy	blaNDM-5	21098725F502X6	1064	4127	2242	2242	2247	0	2876	4063	4070
Italy	blaOXA-48	21102457L202X6	2215	5051	2610	2610	2617	2876	0	5029	5036
Italy	blaOXA-181	21094270C326X6	3427	3068	4856	4856	4861	4063	5029	0	73
Italy	blaOXA-181	21112463I110X6	3434	3073	4859	4859	4864	4070	5036	73	0

CRITERIA FOR THE MSs/RCs ON THE ACTIONS TO BE DONE BASED ON THE RESULTS OF THE CONFIRMATORY TESTING EXERCISE

Confirmatory testing exercise results	Explanation	Action / To be done
1. The results of the confirmatory testing exercise are in concordance between MS/RC and EURL-AR. There is no change in the categorization of the isolate .	<ul style="list-style-type: none"> If there is variability on the MICs reported by the MS/RCs and the MICs from EURL-AR, but no more than one step dilution difference. 	The MSs/RCs will be informed. No additional action is required from the MS/RC.
	<ul style="list-style-type: none"> If there is variability on the MICs reported by the MS/RCs and the MICs from EURL-AR, with more than one step dilution difference. 	The MSs/RCs will be informed. No additional action is required from the MS/RC. The MS can decide if they want to take further actions.

Confirmatory testing exercise results	Explanation	Action / To be done
2. The results indicate a change in the categorization of the isolate.	If the change does not affect the criteria for which the isolate has been selected for the confirmatory testing exercise.	The MSs/RCs will be informed but no additional action will be requested . The MS can decide if they want to take further actions.
	If the change does not affect the criteria for which the isolate has been selected for the confirmatory testing exercise, but the profile of the isolate identified in the confirmatory testing exercise is completely different to the profile reported by the MS/RC.	The EURL-AR retests the isolate once more before going back to the MSs/RCs . If the result is confirmed, then the MSs/RCs will be informed and requested to retest and change the result in EFSA DWH as appropriate.
	If the change does affect the criteria for which the isolate has been selected for the confirmatory testing exercise (*exception TAZ and FOT).	The MSs/RCs informed and requested to retest and change the result in EFSA DWH as appropriate.
	*When the change <u>does affect</u> the resistance/susceptibility to TAZ and FOT: If there is <u>only a difference</u> of one-step dilution.	The MSs/RCs informed; no additional/further action will be requested. The MS can decide if they want to take further actions.
	*When the change <u>does affect</u> the resistance/susceptibility to TAZ and FOT: If there is a difference of <u>more than</u> one-step dilution.	The MSs/RCs informed and requested to retest and change the result in EFSA DWH as appropriate.

MS: Member State; **RC:** Reporting Country; **DWH:** Data Warehouse, **EURL-AR:** European Union Reference Laboratory on Antimicrobial Resistance

The 2021 confirmatory testing is finalised thanks to ...

- The collaboration of all NRLs.
- Our lab technicians at DTU.
- Birthe - for all the lab work and her involvement in managing the confirmatory testing.
- Natasia Rebekka Thornval for the bioinformatic screening, data extraction, preparing the phenotype-genotype concordance table.
- Jette and Rene for their help in the phenotype-genotype interpretation.

THANK YOU FOR YOUR ATTENTION