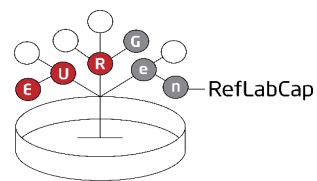


DTU



Results from the third External Quality Assessment (EQA) exercise



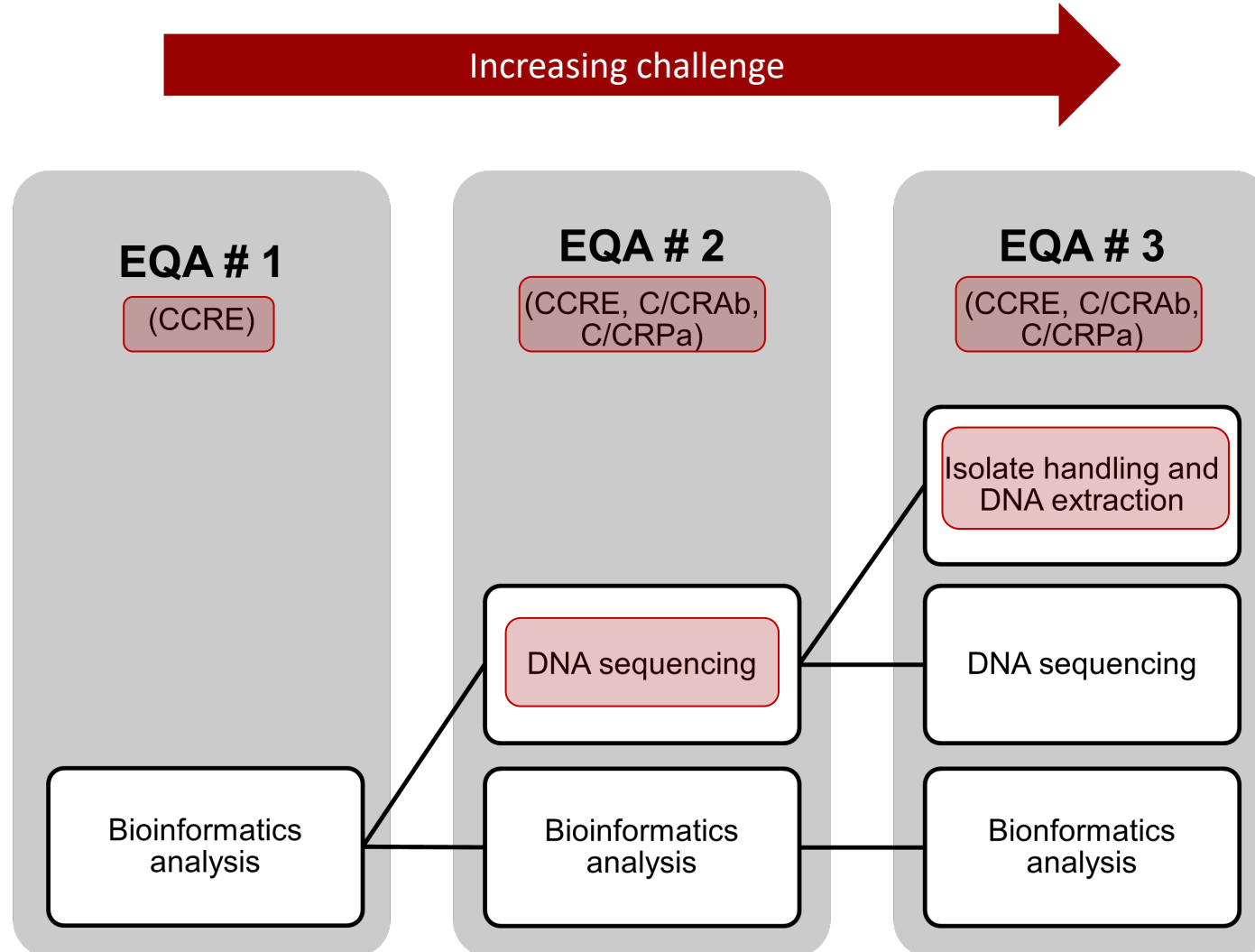
EURGen-RefLabCap

Faisal Ahmad Khan (fakh@food.dtu.dk)

INTRODUCTION

- OVERVIEW OF THE EURGen-RefLabCap EQAS
- DESIGN OF THE SECOND EQA
- PREPARATION OF EXPECTED RESULTS
- SCORING SYSTEM

OVERVIEW OF ALL EURGen-RefLabCap EQAs



- Workstream 1 pathogens (WS1)
 - CRE/CCRE
- Workstream 2 pathogens (WS2)
 - C/CRPa
 - C/CRAb

DESIGN OF THE THIRD EQA

Strains:

- EURGen-2024-01 (*Escherichia coli*)
- EURGen-2024-02 (*Pseudomonas aeruginosa*)
- EURGen-2024-03 (*Acinetobacter baumannii*)
- EURGen-2024-04 (*Klebsiella pneumoniae*)

Bioinformatics analyses included in EQA 2024:

1. Prediction of MLST
2. Prediction of plasmid replicon types
3. Detection of genes and chromosomal mutations mediating AMR
4. *In silico* prediction of AMR profiles

Materials:

- Purified DNA
- Swabs (bacterial cultures)
- (Raw and assembled reads from Illumina and nanopore sequencing technology)

Additional Analyses

Quality control of sequences generated by participants

- I. *Short-read sequences*
- II. *Long-read sequences*

PREPARATION OF EXPECTED RESULTS

- Consensus results from TWO reference laboratories
 - Sequencing and bioinformatics analysis at DTU
 - Sequencing and bioinformatics analysis at SSI
- Bioinformatics tools used to prepare expected results
 - Mainly CGE tools (MLST, ResFinder, PlasmidFinder)
 - AMRFinder+
 - RGI (CARD database)
 - PathogenWatch

Final set of expected results

Categorical agreement
+
ID $\geq 90\%$
+
Coverage $\geq 90\%$ (plasmids)/ $\geq 60\%$ (ARGs)

Expected non mandatory results

- No consensus between reference labs
- Detection in only one type of dataset (lr or sr)
 - Detection in only one tool

SCORING SYSTEM IN THE WEBTOOL

Analysis	Submitted result	Score
Prediction of MLST	Correct MLST	1
	Incorrect MLST	0
Detection of plasmid replicons, AMR genes and chromosomal mutations	Genetic determinant correctly identified	1
	Reporting a genetic determinant that was part of the expected results but not mandatory to report	blank
	Missing a genetic determinant	blank
	Reporting an unexpected genetic determinant	0
<i>In-silico</i> AMR profiles	AMR profile correctly reported for the antimicrobial	1
	Reporting an antimicrobial that was part of the expected results but not mandatory to report, or part of intrinsic resistance	blank
	Missing an antimicrobial	blank
	Reporting an AMR profile for an unexpected antimicrobial	0

SCORING SYSTEM

Maximum possible score of participants

Material and analysis	EURGen-2024-01	EURGen-2024-02	EURGen-2024-03	EURGen-2024-04	Total
Prediction of ST	1	1	1	1	4
Detection of plasmid replicons	5	0	0	6	11
Detection of AMR genes and chromosomal PMs	16	8	4	13	41
<i>In silico</i> prediction of AMR profiles	12	11	5	16	44
Total	34	20	10	36	100

RESULTS AND DISCUSSION

- MATERIAL ANALYSED BY PARTICIPANTS
- OVERALL SCORES OF THE PARTICIPANTS
- PREDICTION OF MLST
- DETECTION OF PLASMID REPLICATION GENES
- DETECTION OF AMR GENES AND MUTATIONS
- *IN SILICO* PREDICTION OF AMR PROFILES

MATERIAL ANALYSED BY PARTICIPANTS

Total participating laboratories: 31

- WS1: 31 laboratories submitted results
- WS2: 27 laboratories submitted results

	EURGen-2024-01 (<i>E. coli</i>)	EURGen-2024-02 (<i>P. aeruginosa</i>)	EURGen-2024-03 (<i>A. baumannii</i>)	EURGen-2024-04 (<i>K. pneumoniae</i>)
Number of Laboratories	31	27	27	31

Test material used:

- Both BACT and DNA: 21 laboratories
- Only BACT: 4 laboratories
- Only DNA: 2 laboratories
- FASTA/FASTQ: 4 laboratories

Total submissions: 52

OVERALL SCORES OF THE PARTICIPANTS

Averages of scores (%)

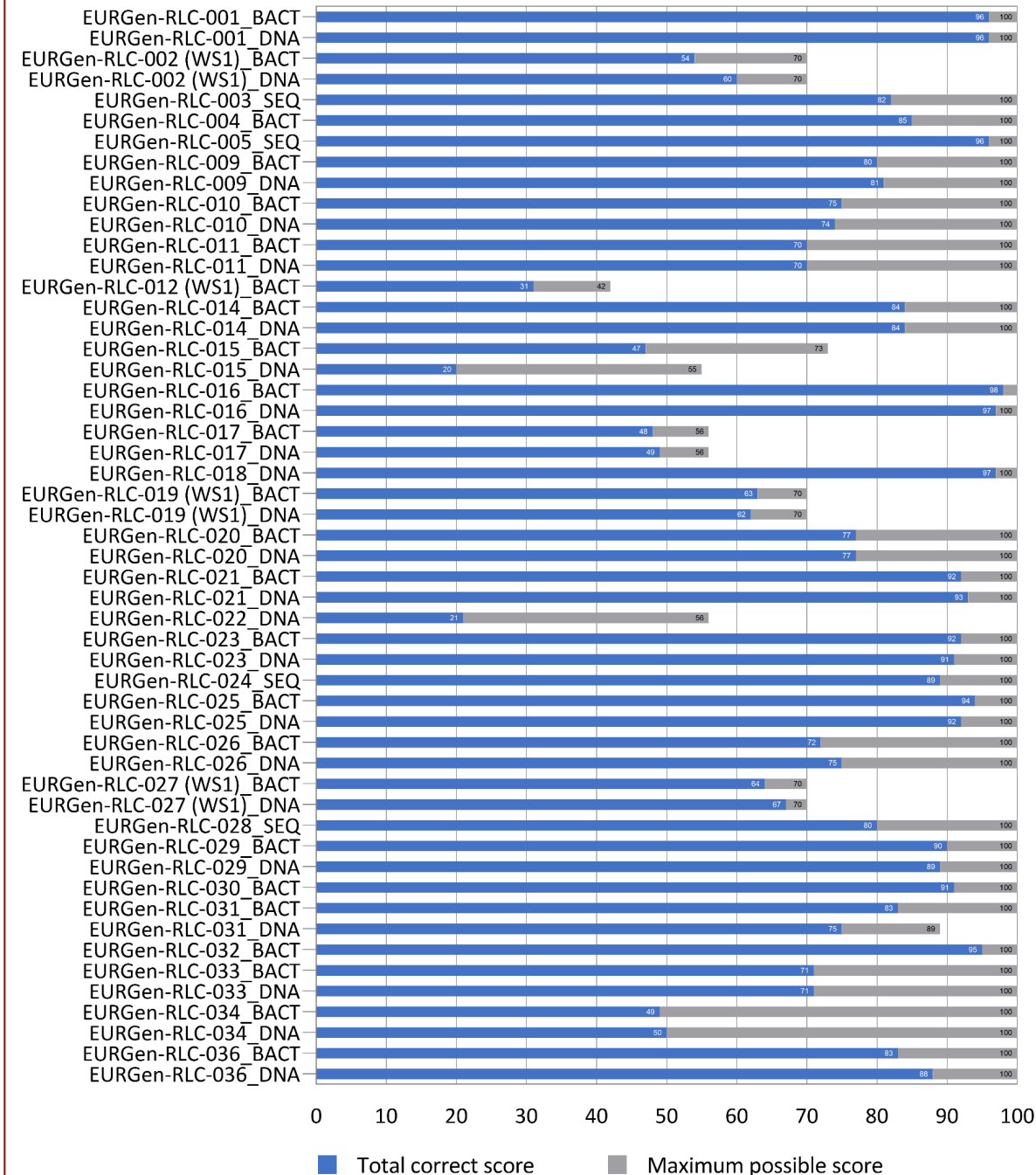
Prediction of MLST: **96%**

Detection of plasmid replicons: **81%**

Prediction of genetic AMR determinants: **79%**

Prediction of AMR profiles: **85%**

Total: 82%



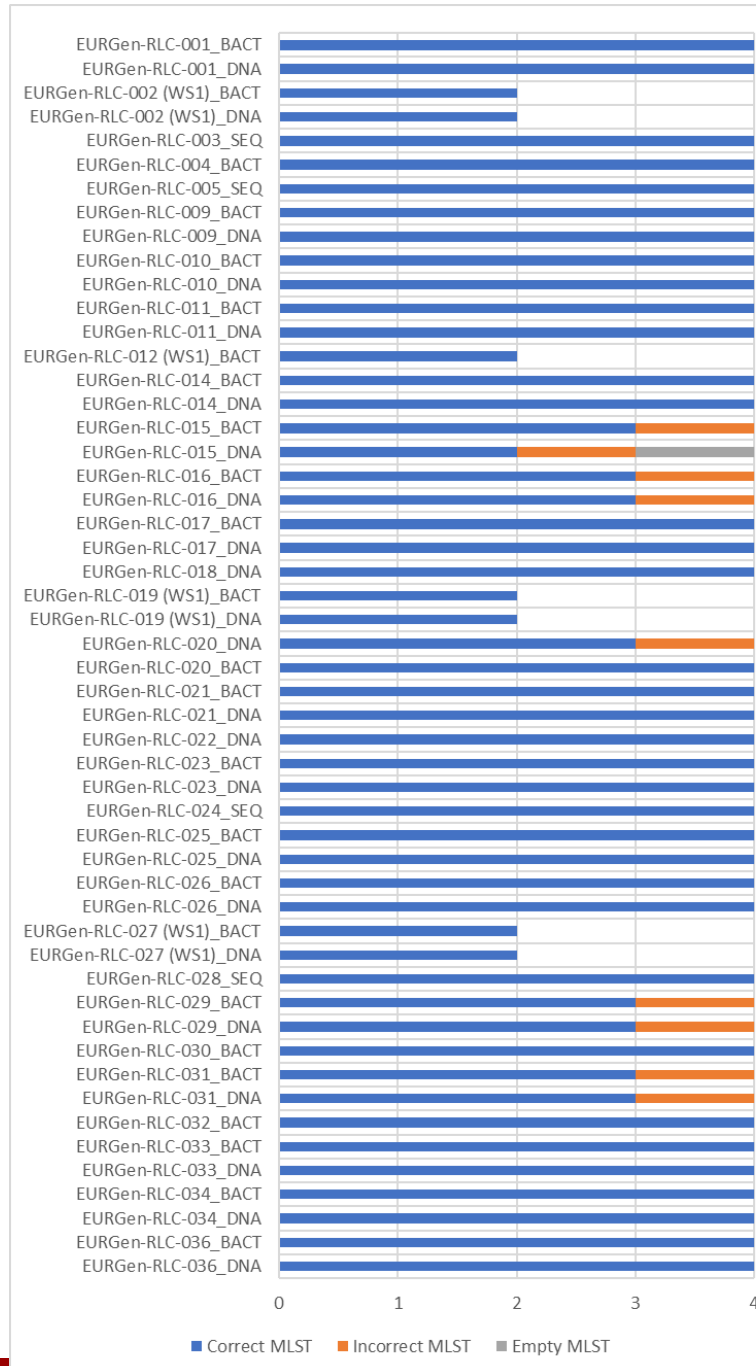
PREDICTION OF MLST – EXPECTED RESULTS

Material	MLST	Alleles assigned to each locus						
EURGen-2024-01 (<i>E. coli</i>)*	457	<i>adk</i>	<i>fumC</i>	<i>gyrB</i>	<i>icd</i>	<i>mdh</i>	<i>purA</i>	<i>recA</i>
		101	88	97	108	26	79	2
EURGen-2024-02 (<i>P. aeruginosa</i>)	644	<i>acsA</i>	<i>aroE</i>	<i>guaA</i>	<i>mutL</i>	<i>nuoD</i>	<i>ppsA</i>	<i>trpE</i>
		28	3	94	13	1	4	10
EURGen-2024-03 (<i>A. baumannii</i>)	1780	<i>cpn60</i>	<i>gdhB</i>	<i>gltA</i>	<i>gpi</i>	<i>gyrB</i>	<i>recA</i>	<i>rpoD</i>
		1	42	1	159	17	12	6
EURGen-2024-04 (<i>K. pneumoniae</i>)*	16	<i>gapA</i>	<i>infB</i>	<i>mdh</i>	<i>pgi</i>	<i>phoE</i>	<i>rpoB</i>	<i>tonB</i>
		2	1	2	1	4	4	4

* For EURGen-2024-01 (*E. coli*), Achtman scheme was used (*E. coli*#1, if using CGE MLST tool). For EURGen-2024-03 (*A. baumannii*), Oxford scheme was used (*A. baumannii*#1, if using CGE MLST tool).

PREDICTION OF MLST - SUBMITTED RESULTS

- Average score : **96%**
- All MLST were correct for EURGen-2024-01, EURGen-2024-02 and EURGen-2024-04
- 9 Incorrect MLST
 - All for EURGen-2024-03 (*A. baumannii*)



PREDICTION OF MLST - DISCUSSION

09 Incorrect MLST results for *A. baumannii* (EURGen-2024-03)

- Due to MLST scheme used
 - These participants used Pasteur scheme (ST 764)
 - Oxford scheme used for expected results (ST 1780)

- For the self-evaluation, it should be considered that these discrepancies do not represent a flaw in the bioinformatics analysis performed
- It is important to understand that the bioinformatics capacity and knowledge required for using either MLST scheme is the same

DETECTION OF PLASMID REPLICONS – EXPECTED RESULTS

Material	Plasmid replicons	Nr.
EURGen-2024-01 (<i>E.coli</i>)	Expected: ColpEC648, IncFIA, IncFIB(AP001918), IncFII, IncI1-I(Alpha)	5
EURGen-2024-02 (<i>P. aeruginosa</i>)	No plasmid replicon detected	0
EURGen-2024-03 (<i>A. baumannii</i>)	No plasmid replicon detected	0
EURGen-2024-04 (<i>K. pneumoniae</i>)	Expected: Col440II, IncFIB(K), IncFII, IncFII(K), IncN4, IncX3	6
	Expected but non-mandatory: Col(pHAD28), ColKP3	2

DETECTION OF PLASMID REPLICONS - SUBMITTED RESULTS

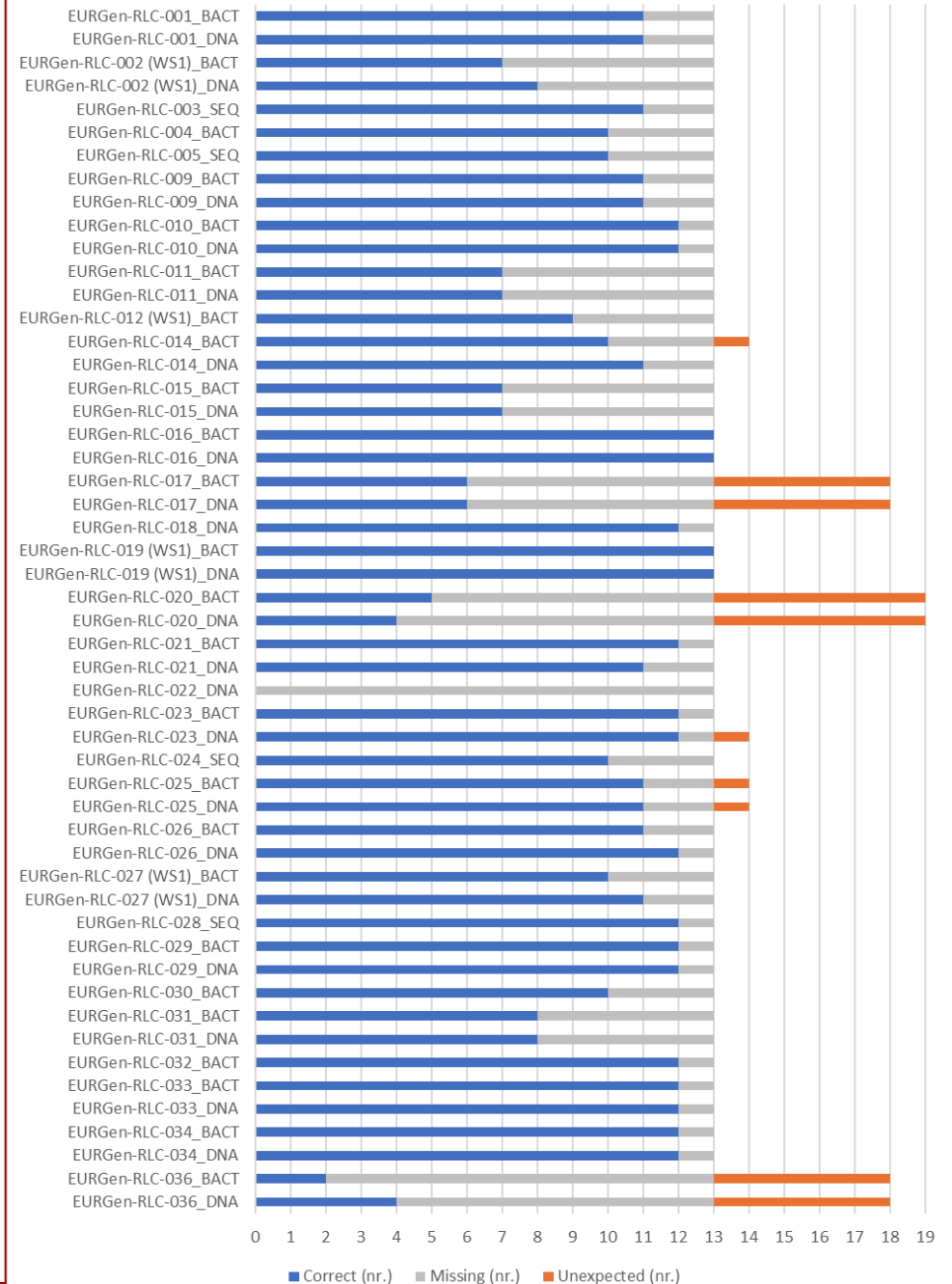
Total submissions: 103

- 50% submissions were fully correct (n=51)
 - 33% had missing replicons (n=34)
 - 17.5% had unexpected replicons (n=18)
- 16.5% Simultaneous (n=17)

Average score: 81%

- 21 submissions achieved 100% of max. possible score

Clarification on the data in the figure: Each bar represents submissions for all isolates for a particular sample type by a laboratory. For example, EURGen-RLC-001_BACT represents submissions for all isolates (n=2 in this case) by laboratory EURGen-RLC-001 for the BACT sample.



DETECTION OF PLASMID REPLICONS - SUBMITTED RESULTS

Strain	Missing expected replicons	Unexpected replicons
EURGen-2024-01	ColpEC648 (n=16)	IncFIC(FII) (n=8)
	IncFII (n=16)	Incl(Gamma) (n=6)
	Incl1-I(Alpha) (n=8)	
	IncFIB(AP001918)(n=5)	
EURGen-2024-04	Col(pHAD28)* (n=48)	ColRNAI (n=6)
	colKP3* (n=19)	IncFIA (n=6)
	IncFII(K) (n=22)	IncFIC(FII) (n=6)
	IncN4 (n=16)	IncN (n=6)
	Col440II (n=10)	

* Expected but non-mandatory

DETECTION OF PLASMID REPLICONS - DISCUSSION

- The non-mandatory replicons were missing in most results
 - Col(pHAD28) (n=48) and ColKP3 (n=19) were only detected in raw reads while preparing the expected results



Discrepancy between short-and long-read data:

- long-read sequencing is overall more adequate for detection of plasmids
- the assembly process might fail to properly capture sequences that were present in raw data

- The missing plasmid replicons:
 - Choice of different thresholds (?)



Different approaches according to purpose:

- Thresholds can be adjusted for different analyses
- Perhaps better to be less strict and manually evaluate results

DETECTION OF GENES AND MUTATIONS MEDIATING AMR – EXPECTED RESULTS

Material	AMR genes and chromosomal mutations	Nr.
EURGen-2024-01 (<i>E. coli</i>)	Expected: <i>aac(3)-IIa^a</i> , <i>bla_{CTX-M-65}</i> , <i>bla_{TEM-1}^b</i> , <i>dfrA12</i> , <i>dfrA17</i> , <i>sul1</i> , <i>sul2</i> , <i>sul3</i> , <i>fosA3</i> , <i>mcr-1.1^c</i> , <i>glpT</i> E448K, <i>gyrA</i> D87Y, <i>gyrA</i> S83L, <i>parC</i> S80I, <i>parE</i> S458A, <i>uhpT</i> E350Q	16
EURGen-2024-02 (<i>P. aeruginosa</i>)	Expected: <i>aph(3')-VI^d</i> , <i>aac(6')-Ib^e</i> , <i>bla_{IMP-62}</i> , <i>bla_{NDM-1}</i> , <i>bla_{PME-1}</i> , <i>crpP</i> , <i>qnrVC1</i> , <i>gyrA</i> T83I	8
	Expected but non-mandatory: <i>ant(3'')-Ii-aac(6')-Iid^f</i> , <i>aac(6')-Ib-cr^g</i> , <i>bla_{KBL-1}</i> , <i>qepA^h</i> , <i>nalC</i> G71E, <i>nalC</i> S209R, <i>parC</i> S87L	7
EURGen-2024-03 (<i>A. baumannii</i>)	Expected: <i>ant(2'')-Ia</i> , <i>bla_{OXA-23}</i> , <i>gyrA</i> S81L, <i>parC</i> S84L	4
	Expected but non-mandatory: <i>parC</i> D105E, <i>parC</i> V104I	2
EURGen-2024-04 (<i>K. pneumoniae</i>)	Expected: <i>aac(6')-Ib-crⁱ</i> , <i>bla_{CTX-M-15}^j</i> , <i>bla_{NDM-5}</i> , <i>bla_{OXA-1}</i> , <i>bla_{OXA-181}</i> , <i>bla_{TEM-1}^b</i> , <i>dfrA12</i> , <i>qnrS1</i> , <i>rmtB^k</i> , <i>sul1</i> , <i>gyrA</i> D87N, <i>gyrA</i> S83F, <i>parC</i> E84K	13
	Expected but non-mandatory: <i>mgrB</i> W20R	1

^a Either *aac(3)-IIa* or *aac(3)-IIE*

^b Either *bla_{TEM-1}* or *bla_{TEM-1A}* or *bla_{TEM-1B}* or *bla_{TEM-1C}* or *bla_{TEM-1D}*

^c Either *mcr1.1* or *mcr-1.26*

^d Either *aph(3')-VI* or *aph(3')-Via*

^e Either *aac(6')-Ib* or *aac(6')-Ib-Hangzhou* or *aac(6')-Ib3* or *aac(6')-Ib4* or *aac(6')-Ib9*

^f Either *ant(3'')-Ii-aac(6')-Iid* or *ant(3'')-Ih/aac(6')-Iid*

^g Either *aac(6')-Ib-cr* or *aac(6')-Ib-cr5*

^h Either *qepA* or *qepA1* or *qepA2* or *qepA4*

ⁱ Either *aac(6')-Ib-cr* or *aac(6')-Ib-cr5* or *aac(6')-Ib-cr6*

^j Either *bla_{CTX-M-15}* or *bla_{CTX-M-101}*

^k Either *rmtB* or *rmtB1*

DETECTION OF GENES AND MUTATIONS – SUBMITTED RESULTS

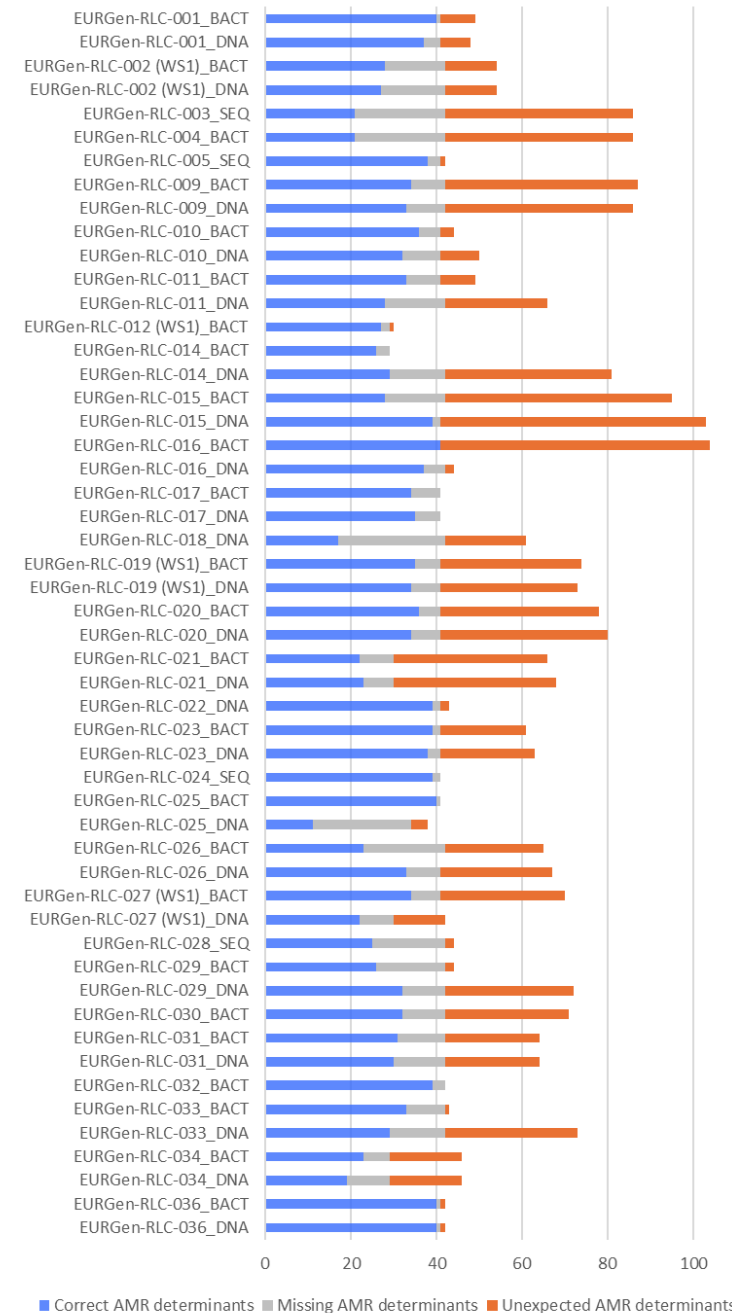
Total submissions: 192

- 9.9% submissions were fully correct (n=19)
 - 17% had missing AMR determinants (n=33)
 - 73% had unexpected determinants (n=140)
- 54% Simultaneous (n=103)

Average score: 79%

- None of the participants achieved 100% of their max. possible score
- Highest achieved score was 97.5%

Clarification on the data in the figure: Each bar represents submissions for all isolates for a particular sample type by a laboratory. For example, EURGen-RLC-001_BACT represents submissions for all isolates (n=4 in this case) by laboratory EURGen-RLC-001 for the BACT sample.



DETECTION OF GENES AND MUTATIONS – DISCUSSION

Strain	Examples of problems
01	Missing <i>glpT</i> E448K (n=26) or <i>uhpT</i> E350Q (n=27)
	Unexpected <i>aadA1</i> (n=23), <i>aadA2</i> (n=25), <i>aadA5</i> (n=24), <i>tet(A)</i> (n=20), <i>tet(M)</i> (n=16)
02	Missing <i>aac(6')</i> (n=19), <i>crpP</i> (n=18)
	Unexpected <i>bla</i> _{PAO} (n=10), <i>bla</i> _{PCD} (n=17), <i>bla</i> _{OXA-486} (n=18), <i>bla</i> _{CARB-2} (n=23), <i>fosA</i> (n=25), <i>aph(3')-Ib</i> (n=18)
	Unexpected <i>tet(G)</i> (n=15), <i>aadA</i> (n=13), <i>aadA2</i> (n=12)
03	Missing <i>gyrA</i> S81L (n=19), <i>parC</i> S84L (n=19)
	Unexpected, <i>bla</i> _{OXA-429} (n=23), <i>bla</i> _{CARB-2} (n=25), <i>bla</i> _{PDC-262} (n=16)
	<i>aph(3'')-Ib</i> (n=19), <i>sul2</i> (n=14), <i>tet(B)</i> (n=18), <i>tet(G)</i> (n=18), <i>aadA2</i> (n=19)
04	Missing <i>gyrA</i> D87N (n=21), <i>parC</i> E84K (n=23), <i>gyrA</i> S83F (n=21)
	Unexpected <i>fosA/fosA5</i> (n=21), <i>oqxA/oqxB</i> (n=18)
	Unexpected <i>aph(3')-Ia</i> (n=24), <i>aadA2</i> (n=26)

Missing mutations due to lacking database

- PointFinder can't detect PMs in *A. baumannii* and *P. aeruginosa* – No database!
- *glpT*, *uhpT* mutations not present in PointFinder database (ResFinder)
- Similarly, *gyrA* and *parC* mutations were missing for *A. baumannii*



Multiple tools and database can be used:

- AMRFinder+
- CARD
- ResFinder
- Other tools (PathogenWatch?)

DETECTION OF GENES AND MUTATIONS – DISCUSSION

Expected mutations were reported incorrectly

- Many participants reported the expected PMs but in different format than described in EQA protocol
 - E.g., *gyrA* D87Y
- The mutations are present in the isolate

EURGen-2024-001

<i>gyrA</i> p.D87Y
<i>gyrA</i> p.S83L
<i>gyrA</i> S83L;D87Y
<i>parC</i> p.S80I
<i>parC</i> parC_S80I
<i>parE</i> p.S458A
<i>parE</i> parE_S458A



- For the self-evaluation, it should be considered that these discrepancies do not represent a flaw in the bioinformatics analysis performed

DETECTION OF GENES AND MUTATIONS – DISCUSSION

Strain	Examples of problems
01	Missing <i>glpT</i> E448K (n=26) or <i>uhpT</i> E350Q (n=27)
	Unexpected <i>aadA1</i> (n=23), <i>aadA2</i> (n=25), <i>aadA5</i> (n=24), <i>tet(A)</i> (n=20), <i>tet(M)</i> (n=16)
02	Missing <i>aac(6')</i> (n=19), <i>crpP</i> (n=18)
	Unexpected <i>bla</i> _{PAO} (n=10), <i>bla</i> _{PCD} (n=17), <i>bla</i> _{OXA-486} (n=18), <i>bla</i> _{CARB-2} (n=23), <i>fosA</i> (n=25), <i>aph(3')-Ib</i> (n=18)
	Unexpected <i>tet(G)</i> (n=15), <i>aadA</i> (n=13), <i>aadA2</i> (n=12)
03	Missing <i>gyrA</i> S81L (n=19), <i>parC</i> S84L (n=19)
	Unexpected, <i>bla</i> _{OXA-429} (n=23), <i>bla</i> _{CARB-2} (n=25), <i>bla</i> _{PDC-262} (n=16)
	<i>aph(3'')-Ib</i> (n=19), <i>sul2</i> (n=14), <i>tet(B)</i> (n=18), <i>tet(G)</i> (n=18), <i>aadA2</i> (n=19)
04	Missing <i>gyrA</i> D87N (n=21), <i>parC</i> E84K (n=23), <i>gyrA</i> S83F (n=21)
	Unexpected <i>fosA/fosA5</i> (n=21), <i>oqxA/oqxB</i> (n=18)
	Unexpected <i>aph(3')-Ia</i> (n=24), <i>aadA2</i> (n=26)

Reporting intrinsic genes

- Present in the strains but do not contribute to the elevated resistance in non-WT phenotype



Results must be evaluated critically:

- Too much noise can hide the important information
- Insufficient knowledge regarding genetic mechanisms of AMR might lead to incorrect reporting of resistance profiles

DETECTION OF GENES AND MUTATIONS – DISCUSSION

Strain	Examples of problems
01	Missing <i>glpT</i> E448K (n=26) or <i>uhpT</i> E350Q (n=27)
	Unexpected <i>aadA1</i> (n=23), <i>aadA2</i> (n=25), <i>aadA5</i> (n=24), <i>tet(A)</i> (n=20), <i>tet(M)</i> (n=16)
02	Missing <i>aac(6')</i> (n=19), <i>crpP</i> (n=18)
	Unexpected <i>bla</i> _{PAO} (n=10), <i>bla</i> _{PCD} (n=17), <i>bla</i> _{OXA-486} (n=18), <i>bla</i> _{CARB-2} (n=23), <i>fosA</i> (n=25), <i>aph(3')-Ib</i> (n=18)
	Unexpected <i>tet(G)</i> (n=15), <i>aadA</i> (n=13), <i>aadA2</i> (n=12)
03	Missing <i>gyrA</i> S81L (n=19), <i>parC</i> S84L (n=19)
	Unexpected, <i>bla</i> _{OXA-429} (n=23), <i>bla</i> _{CARB-2} (n=25), <i>bla</i> _{PDC-262} (n=16)
	<i>aph(3'')-Ib</i> (n=19), <i>sul2</i> (n=14), <i>tet(B)</i> (n=18), <i>tet(G)</i> (n=18), <i>aadA2</i> (n=19)
04	Missing <i>gyrA</i> D87N (n=21), <i>parC</i> E84K (n=23), <i>gyrA</i> S83F (n=21)
	Unexpected <i>fosA/fosA5</i> (n=21), <i>oqxA/oqxB</i> (n=18)
	Unexpected <i>aph(3')-Ia</i> (n=24), <i>aadA2</i> (n=26)

Reporting AMR genes for antimicrobials not included in EQA

- These genes are present in the strains but they confer resistance to antimicrobials not relevant for the species
- Streptomycin (*aadA1*, *aadA2*, *aadA5*, *aph(6)-Id*, *aph(3'')-Ib*) and Kanamycin (*aph(3')-IIb*), Tetracycline (*tet(A)*, *tet(M)*)



Results must be evaluated critically:

- Too much noise can hide the important information
- Insufficient knowledge regarding genetic mechanisms of AMR might lead to incorrect reporting of resistance profiles

IN SILICO PREDICTION OF AMR PROFILES – EXPECTED RESULTS

Material	Associated prediction of AMR profiles	Nr.
EURGen-2024-01 (<i>E. coli</i>)	Expected: Ampicillin, Aztreonam, Cefepime, Cefotaxime, Ceftazidime, Ciprofloxacin, Colistin, Fosfomycin, Gentamicin, Sulfamethoxazole, Tobramycin, Trimethoprim	12
EURGen-2024-02 (<i>P. aeruginosa</i>)	Expected: Amikacin, Aztreonam, Cefepime, Ceftazidime, Ceftazidime-avibactam, Ciprofloxacin, Gentamicin, Imipenem, Meropenem, Piperacillin-tazobactam, Tobramycin	11
EURGen-2024-03 (<i>A. baumannii</i>)	Expected: Ciprofloxacin, Gentamicin, Imipenem, Meropenem, Tobramycin	5
	Intrinsic*: Aztreonam, fosfomycin	2
EURGen-2024-04 (<i>K. pneumoniae</i>)	Expected: Amikacin, Amoxicillin-clavulanic acid, Aztreonam, Cefepime, Cefotaxime, Ceftazidime, Ceftazidime-avibactam, Ciprofloxacin, Ertapenem, Gentamicin, Imipenem, Meropenem, Piperacillin-tazobactam, Sulfamethoxazole, Tobramycin, Trimethoprim	16
	Expected non-mandatory: Colistin ^a	1
	Intrinsic*: Ampicillin	1

^a Detection of *mgrB* W20R mutation, and subsequent inclusion of colistin in AMR profile of this strain, were expected results, but not mandatory to report

* Intrinsic resistance (based on EUCAST Expected Phenotypes Version 1.2, January 2023), not part of the expected results

IN SILICO PREDICTION OF AMR PROFILES – EXPECTED RESULTS

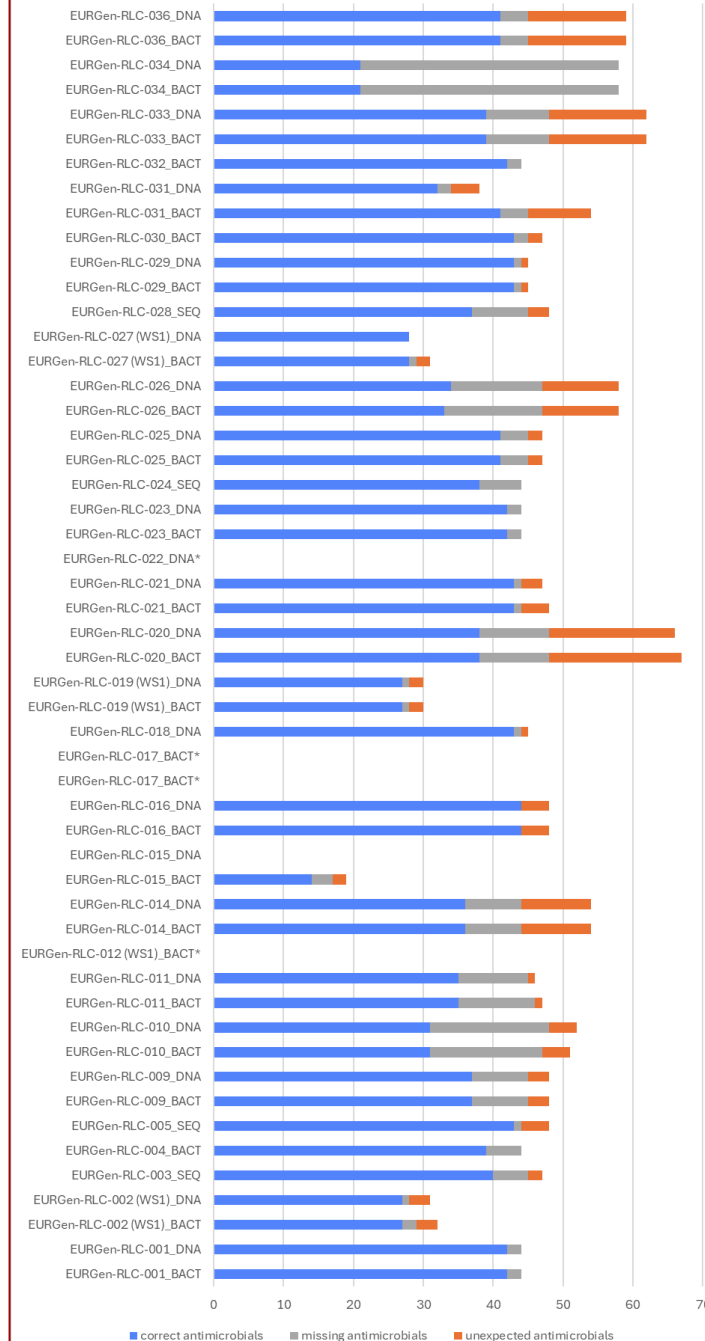
Total submissions: 173

- 24% submissions were fully correct (n=41)
 - 32% had missing antimicrobials (n=55)
 - 44% had unexpected antimicrobials (n=77)
- } 32% Simultaneous (n=56%)

Average score: 85%

- 3 participants achieved 100% of their max. possible score

Clarification on the data in the figure: Each bar represents submissions for all isolates for a particular sample type by a laboratory. For example, EURGen-RLC-001_BACT represents submissions for all isolates (n=4 in this case) by laboratory EURGen-RLC-001 for the BACT sample.



IN SILICO PREDICTION OF AMR PROFILES – SUBMITTED RESULTS

Laboratories	Sample type	Expected												Unexpected			Correct (nr.)	Missing (nr.)	Unexpected (nr.)
		Ampicillin	Aztreonam	Cefepime	Cefotaxime	Ceftazidime	Ciprofloxacin	Colistin	Fosfomycin	Gentamicin	Sulfamethoxazole	Tobramycin	Trimethoprim	Amikacin	Ertapenem	Tigecycline			
EURGen-RLC-001	BACT	x	x	x	x	x	x	x	x	x	x	x	x				12	0	0
	DNA	x	x	x	x	x	x	x	x	x	x	x	x				12	0	0
EURGen-RLC-002	BACT	x	x	x	x	-	x	x	x	x	x	x	x				11	1	0
	DNA	x	x	x	x	-	x	x	x	x	x	x	x				11	1	0
EURGen-RLC-003 ^b	SEQ	x	-	x	x	x	x	x	x	x	x	x	x				11	1	0
EURGen-RLC-004 ^c	BACT	x	x	x	x	x	x	x	x	x	x	x	x				12	0	0
EURGen-RLC-005 ^b	SEQ	x	x	x	x	x	x	x	x	x	x	x	x				12	0	0
EURGen-RLC-009	BACT	x	-	x	x	x	x	x	x	x	x	x	x				11	1	0
	DNA	x	-	x	x	x	x	x	x	x	x	x	x				11	1	0
EURGen-RLC-010	BACT	x	x	x	x	x	-	x	x	x	x	x	x				11	1	0
	DNA	x	x	x	x	x	-	x	x	x	x	x	x				11	1	0
EURGen-RLC-011	BACT	x	-	x	x	x	-	x	x	x	x	x	x				10	2	0
	DNA	x	-	x	x	x	-	x	x	x	x	x	x				10	2	0
EURGen-RLC-014	BACT	-	-	x	x	x	x	x	x	x	x	x	x				10	2	0
	DNA	-	-	x	x	x	x	x	x	x	x	x	x				10	2	0
EURGen-RLC-015	BACT	x	-	x	x	x	x	x	x	-	x	x	x				10	2	0
EURGen-RLC-016	BACT	x	x	x	x	x	x	x	x	x	x	x	x				12	0	0
	DNA	x	x	x	x	x	x	x	x	x	x	x	x				12	0	0
EURGen-RLC-018 ^c	DNA	x	x	x	x	x	x	x	x	x	x	x	x				12	0	0
EURGen-RLC-019	BACT	x	x	x	x	x	-	x	x	x	x	x	x				11	1	0
	DNA	x	x	x	x	x	-	x	x	x	x	x	x				11	1	0
EURGen-RLC-020	BACT	x	x	x	x	x	x	x	x	-	-	x		x	x		10	2	2
	DNA	x	x	x	x	x	x	x	x	-	-	x		x	x		10	2	2
EURGen-RLC-021	BACT	x	x	x	x	x	x	x	x	x	x	x	x				12	0	0
	DNA	x	x	x	x	x	x	x	x	x	x	x	x				12	0	0
EURGen-RLC-023	BACT	x	x	x	x	x	x	x	x	x	x	x	x				12	0	0
	DNA	x	x	x	x	x	x	x	x	x	x	x	x				12	0	0
EURGen-RLC-024 ^b	SEQ	x	x	x	x	x	x	x	-	x	-	x					10	2	0
EURGen-RLC-025	BACT	x	x	x	x	x	x	x	x	x	x	x	x				12	0	0
	DNA	x	x	x	x	x	x	x	x	x	x	x	x				12	0	0
EURGen-RLC-026	BACT	-	-	x	x	x	-	x	-	x	-	x					7	5	0
	DNA	-	-	x	x	x	-	x	x	x	-	x					8	4	0
EURGen-RLC-027	BACT	x	x	x	x	x	x	x	x	x	x	x	x				12	0	0
	DNA	x	x	x	x	x	x	x	x	x	x	x	x				12	0	0
EURGen-RLC-028 ^b	SEQ	x	-	x	x	x	x	x	x	x	x	x	x				11	1	0
EURGen-RLC-029	BACT	x	-	x	x	x	x	x	x	x	x	x	x				11	1	0
	DNA	x	-	x	x	x	x	x	x	x	x	x	x				11	1	0
EURGen-RLC-030 ^c	BACT	x	-	x	x	x	x	x	x	x	x	x	x				11	1	0
EURGen-RLC-031	BACT	x	x	x	x	x	x	x	x	x	x	x	x				12	0	0
	DNA	x	x	x	x	x	x	x	x	x	x	x	x				12	0	0
EURGen-RLC-032 ^c	BACT	x	-	x	x	x	x	x	x	x	-	x	x				11	1	0
EURGen-RLC-033	BACT	x	x	x	x	x	x	x	x	-	-	x		x			10	2	1
	DNA	x	x	x	x	x	x	x	x	-	-	x		x			10	2	1
EURGen-RLC-034	BACT	-	-	-	-	-	-	x	-	-	-	-					1	11	0
	DNA	-	-	-	-	-	-	x	-	-	-	-					1	11	0
EURGen-RLC-036	BACT	x	-	x	x	x	x	x	x	x	x	x	x				11	1	0
	DNA	-	x	x	x	x	x	x	x	x	x	x	x				11	1	0
Correct (nr.)		40	29	45	45	43	37	47	45	43	40	38	45	NA	NA	NA		Total	
Missing or UN (nr.)		7	18	2	2	4	10	0	2	4	7	9	2	2	2	2	497	67	6

Strain EURGen-2024-01 (*E. coli*)

- Missing from most submitted results
 - Aztreonam (n=18)
 - Only ResFinder reports Aztronam for the R-profile of *bla*_{CTX-M-65}
 - Ciprofloxacin (n=10)
 - Missing *gyrA* and *parC* mutations in some cases

Results must be evaluated critically:

- Results can / should be confirmed with other tools

IN SILICO PREDICTION OF AMR PROFILES – SUBMITTED RESULTS

Strain EURGen-2024-02 (*P. aeruginosa*)

- Missing from most submitted results

- Aztreonam (n=27)

- Missing from bla_{PME} R-profile in all three tools

- Ceftazidime-avibactam (n=22)

- Missing from bla_{NDM-1} and bla_{IMP-62} R-profile in ResFinder database

- Tobramycin (n=19)

- Missing in AMRFinder+ for the aminoglycoside resistance genes ($aac(6')-Ib$ family)

- Reporting of intrinsic resistance

- Fosfomycin (n=20)

- $fosA$ is intrinsic in *P. aeruginosa*

Results must be evaluated critically:

- Results can / should be confirmed with other tools
- Points to insufficient knowledge of genetic AMR mechanisms

Laboratories	Sample type	Expected											Unexpected							Correct (nr.)	Missing (nr.)	Unexpected (nr.)
		Amikacin	Aztreonam	Cefepime	Ceftazidime	Ceftazidime-avibactam	Ciprofloxacin	Gentamicin	Imipenem	Meropenem	Piperacillin-tazobactam	Tobramycin	Amoxicillin-clavulanic acid	Ampicillin	Cefotaxime	Ertapenem	Fosfomycin	Sulfamethoxazole	Tigecycline			
EURGen-RLC-001	BACT	x	-	x	x	x	x	-	x	x	x	x								9	2	0
	DNA	x	-	x	x	x	x	-	x	x	x	x								9	2	0
EURGen-RLC-003 ^b	SEQ	x	-	x	x	-	x	x	x	x	x	x					x			9	2	1
EURGen-RLC-004 ^c	BACT	-	-	x	x	x	x	-	x	x	x	-								7	4	0
EURGen-RLC-005 ^b	SEQ	x	-	x	x	x	x	x	x	x	x	x								10	1	0
EURGen-RLC-009	BACT	-	-	x	x	-	x	-	x	x	x	-					x			6	5	1
	DNA	-	-	x	x	-	x	-	x	x	x	-					x			6	5	1
EURGen-RLC-010	BACT	x	-	-	-	-	-	x	-	-	-	-					x		x	2	9	2
	DNA	x	-	-	-	-	-	x	-	-	-	-					x		x	2	9	2
EURGen-RLC-011	BACT	-	-	x	x	-	x	-	x	x	x	-								6	5	0
	DNA	-	-	x	x	-	x	-	x	x	x	-								6	5	0
EURGen-RLC-014	BACT	-	-	x	x	x	-	-	x	x	x	-	x	x	x	x	x		x	6	5	6
	DNA	-	-	x	x	x	-	-	x	x	x	-	x	x	x	x	x		x	6	5	6
EURGen-RLC-016	BACT	x	x	x	x	x	x	x	x	x	x	x								11	0	0
	DNA	x	x	x	x	x	x	x	x	x	x	x								11	0	0
EURGen-RLC-018 ^c	DNA	x	-	x	x	x	x	x	x	x	x	x								10	1	0
EURGen-RLC-020	BACT	x	x	x	x	-	-	x	x	x	-	-	x	x	x	x	x		x	7	4	5
	DNA	x	x	x	x	-	-	x	x	x	-	-	x	x	x	x	x		x	7	4	5
EURGen-RLC-021	BACT	x	x	x	x	x	x	x	x	x	x	x					x			11	0	1
	DNA	x	x	x	x	x	x	x	x	x	x	x					x			11	0	1
EURGen-RLC-023	BACT	x	-	x	x	x	x	x	x	x	x	x								10	1	0
	DNA	x	-	x	x	x	x	x	x	x	x	x								10	1	0
EURGen-RLC-024 ^b	SEQ	-	x	x	x	-	x	-	x	x	x	-								7	4	0
EURGen-RLC-025	BACT	x	-	x	x	-	x	x	x	x	x	x					x			9	2	1
	DNA	x	-	x	x	-	x	x	x	x	x	x					x			9	2	1
EURGen-RLC-026	BACT	-	-	x	x	-	x	x	x	x	x	-	x	x	x	x	x	x		7	4	7
	DNA	-	-	x	x	-	x	x	x	x	x	-	x	x	x	x	x	x		7	4	7
EURGen-RLC-028 ^b	SEQ	-	-	x	x	-	x	-	x	x	x	-								6	5	0
EURGen-RLC-029	BACT	x	x	x	x	x	x	x	x	x	x	x								11	0	0
	DNA	x	x	x	x	x	x	x	x	x	x	x								11	0	0
EURGen-RLC-030 ^c	BACT	x	x	x	x	x	x	x	x	x	x	x								11	0	0
EURGen-RLC-031	BACT	x	-	x	x	-	x	x	x	x	x	x	x	x	x	x	x			9	2	5
EURGen-RLC-032 ^c	BACT	x	-	x	x	x	x	x	x	x	x	x								10	1	0
EURGen-RLC-033	BACT	x	x	x	x	-	x	x	x	x	-	-	x	x	x	x	x		x	8	3	6
	DNA	x	x	x	x	-	x	x	x	x	-	-	x	x	x	x	x		x	8	3	6
EURGen-RLC-034	BACT	-	-	-	-	-	-	-	x	x	-	-								2	9	0
	DNA	-	-	-	-	-	-	-	x	x	-	-								2	9	0
EURGen-RLC-036	BACT	x	-	x	x	-	x	x	x	x	x	x	x	x	x	x	x	x		9	2	7
	DNA	x	-	x	x	-	x	x	x	x	x	x	x	x	x	x	x	x		9	2	7
Correct (nr.)		26	12	35	35	17	31	26	37	37	31	20	NA	NA	NA	NA	NA	NA	NA	Total		
Missing or UN (nr.)		13	27	4	4	22	8	13	2	2	8	19	9	11	11	11	20	4	12	307	122	78

IN SILICO PREDICTION OF AMR PROFILES – SUBMITTED RESULTS

Strain EURGen-2024-03 (*A. baumannii*)

- Missing from many submitted results
 - Ciprofloxacin (n=23)
 - Due to missing *gyrA* and *parC* mutations (lack of PM database in PointFinder for *A. baumannii*)
- Reporting of Intrinsic resistance
 - Ampicillin (n=14)
 - *bla*_{CARB-2} is intrinsic in *A. baumannii*

Results must be evaluated critically:

- Results can / should be confirmed with other tools
- Points to insufficient knowledge of genetic mechanisms of AMR

Laboratories	Sample type	Expected					Unexpected															Correct (nr.)	Missing (nr.)	Unexpected (nr.)
		Ciprofloxacin	Gentamidin	Imipenem	Meropenem	Tobramycin	Amoxicillin-clavulanic acid	Ampicillin	Aztreonam	Cefepime	Cefotaxime	Ceftazidime	Ceftazidime-avibactam	Colistin	Ertapenem	Fosfomycin	Piperacillin-tazobactam	Sulfamethoxazole	Tigecycline	Trimethoprim				
EURGen-RLC-001	BACT	x	x	x	x	x															5	0	0	
	DNA	x	x	x	x	x															5	0	0	
EURGen-RLC-003 ^b	SEQ	-	x	x	x	x															4	1	0	
EURGen-RLC-004 ^c	BACT	x	-	x	x	x															4	1	0	
EURGen-RLC-005 ^b	SEQ	x	x	x	x	x				x		x	x				x				5	0	4	
EURGen-RLC-009	BACT	-	x	x	x	x															4	1	0	
	DNA	-	x	x	x	x															4	1	0	
EURGen-RLC-010	BACT	-	x	-	-	x														x	2	3	1	
	DNA	-	x	-	-	x														x	2	3	1	
EURGen-RLC-011	BACT	-	-	x	x	x															3	2	0	
	DNA	-	-	x	x	x															3	2	0	
EURGen-RLC-014	BACT	-	x	x	x	x		x												x	4	1	2	
	DNA	-	x	x	x	x		x												x	4	1	2	
EURGen-RLC-015 ^c	BACT	-	x	x	x	x		x												x	4	1	2	
EURGen-RLC-016	BACT	x	x	x	x	x				x		x	x				x				5	0	4	
	DNA	x	x	x	x	x				x		x	x				x				5	0	4	
EURGen-RLC-018 ^c	DNA	x	x	x	x	x							x								5	0	1	
EURGen-RLC-020	BACT	x	x	x	x	x		x	x	x	x	x			x	x				x	5	0	9	
	DNA	x	x	x	x	x		x	x	x	x	x			x	x				x	5	0	8	
EURGen-RLC-021	BACT	-	x	x	x	x															4	1	1	
	DNA	-	x	x	x	x															4	1	1	
EURGen-RLC-023	BACT	-	x	x	x	x															4	1	0	
	DNA	-	x	x	x	x															4	1	0	
EURGen-RLC-024 ^b	SEQ	x	x	x	x	x															5	0	0	
EURGen-RLC-025	BACT	-	x	x	x	x															4	1	0	
	DNA	-	x	x	x	x															4	1	0	
EURGen-RLC-026	BACT	-	x	x	x	-		x												x	3	2	2	
	DNA	-	x	x	x	-		x												x	3	2	2	
EURGen-RLC-028 ^b	SEQ	-	x	x	x	x		x												x	4	1	2	
EURGen-RLC-029	BACT	x	x	x	x	x								x							5	0	1	
	DNA	x	x	x	x	x								x							5	0	1	
EURGen-RLC-030 ^c	BACT	x	x	x	x	x															5	0	0	
EURGen-RLC-031	BACT	-	x	x	x	x		x													4	1	2	
	DNA	-	x	x	x	x		x													4	1	2	
EURGen-RLC-032 ^c	BACT	x	x	x	x	x															5	0	0	
EURGen-RLC-033	BACT	x	x	x	x	x		x	x						x	x					5	0	5	
	DNA	x	x	x	x	x		x	x						x	x					5	0	5	
EURGen-RLC-034	BACT	-	-	x	x	-															2	3	0	
	DNA	-	-	x	x	-															2	3	0	
EURGen-RLC-036	BACT	x	x	x	x	x		x	x						x		x	x			5	0	5	
	DNA	x	x	x	x	x		x	x						x		x	x			5	0	5	
EURGen-RLC-012																					NA	NA	NA	
Correct (nr.)		18	36	39	39	37	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	Total			
Missing or UN (nr.)		23	5	2	2	4	4	14	2	5	2	5	4	2	6	6	5	10	2	5	169	36	72	

Laboratories	Sample type	Expected																	Unexpected			Correct (nr.)	Missing (nr.)	Unexpected (nr.)	Expected non-mandatory	
		Amikacin	Amoxicillin-clavulanic acid	Aztreonam	Cefepime	Cefotaxime	Ceftazidime	Ceftazidime-avibactam	Ciprofloxacin	Ertapenem	Gentamicin	Imipenem	Meropenem	Piperacillin-tazobactam	Sulfamethoxazole	Tobramycin	Trimethoprim	Colistin ^a	Ampicillin	Fosfomycin	Tigecycline					
EURGen-RLC-001	BACT	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x		16	0	0	1	
	DNA	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x		16	0	0	1	
EURGen-RLC-002	BACT	x	x	-	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	15	1	3	1	
	DNA	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	16	0	3	1	
EURGen-RLC-003 ^b	SEQ	x	x	x	x	x	x	-	x	x	x	x	x	x	x	x	x	-		x		15	1	1	0	
EURGen-RLC-004 ^c	BACT	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	-				16	0	0	0	
EURGen-RLC-005 ^b	SEQ	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	-	-			16	0	0	0	
EURGen-RLC-009	BACT	x	x	x	x	x	x	-	x	x	x	x	x	x	x	x	x	-	x	x		15	1	2	0	
	DNA	x	x	x	x	x	x	-	x	x	x	x	x	x	x	x	x	-	x	x		15	1	2	0	
EURGen-RLC-010	BACT	x	x	x	x	x	x	-	x	x	-	x	x	x	x	x	-	-	x			13	3	1	0	
	DNA	x	x	x	x	x	x	-	x	x	-	x	x	-	-	x	-	-	x			12	4	1	0	
EURGen-RLC-011	BACT	x	x	x	x	x	x	-	x	x	x	x	x	-	-	x	-	-	x			14	2	1	0	
	DNA	x	x	x	x	x	x	-	x	x	x	x	x	x	x	x	-	-	x			15	1	1	0	
EURGen-RLC-014	BACT	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	-	x	x		16	0	2	1	
	DNA	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	-	x	x		16	0	2	1	
EURGen-RLC-016	BACT	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	-				16	0	0	1	
	DNA	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	-				16	0	0	1	
EURGen-RLC-018 ^c	DNA	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	-				16	0	0	1	
EURGen-RLC-019	BACT	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	-	x	x		16	0	2	0	
	DNA	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	-	x	x		16	0	2	0	
EURGen-RLC-020	BACT	x	-	x	x	x	x	-	x	x	x	x	-	-	x	x	x	-	x	x	x	12	4	3	1	
	DNA	x	-	x	x	x	x	-	x	x	x	x	-	-	x	x	x	-	x	x	x	12	4	3	1	
EURGen-RLC-021	BACT	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	-	x		x	16	0	2	1	
	DNA	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	-	x			16	0	1	1	
EURGen-RLC-023	BACT	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	-				16	0	0	1	
	DNA	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	-				16	0	0	1	
EURGen-RLC-024 ^b	SEQ	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	-				16	0	0	0	
EURGen-RLC-025	BACT	x	x	x	x	x	x	-	x	x	x	x	x	x	x	x	x	-		x		15	1	1	0	
	DNA	x	x	x	x	x	x	-	x	x	x	x	x	x	x	x	x	-		x		15	1	1	0	
EURGen-RLC-026	BACT	-	x	x	x	x	x	-	x	x	x	x	x	x	-	-	x	-	x	x		13	3	2	0	
	DNA	-	x	x	x	x	x	-	x	x	x	x	x	x	-	-	x	-	x	x		13	3	2	0	
EURGen-RLC-027	BACT	x	x	-	x	x	x	x	x	x	x	x	x	x	x	x	x	-	x	x	x	15	1	2	1	
	DNA	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	-	x			16	0	0	1	
EURGen-RLC-028 ^b	SEQ	x	x	x	x	x	x	-	x	x	x	x	x	x	x	x	x	-	x			15	1	1	0	
EURGen-RLC-029	BACT	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	-				16	0	0	1	
	DNA	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	-				16	0	0	1	
EURGen-RLC-030 ^c	BACT	x	x	-	x	x	x	x	x	x	x	x	x	x	x	x	x	-	x	x		15	1	2	1	
EURGen-RLC-031	BACT	x	x	x	x	x	x	-	x	x	x	x	x	x	x	x	x	-	x	x		15	1	2	0	
	DNA	x	x	x	x	x	x	-	x	x	x	x	x	x	x	x	x	-	x	x		15	1	2	0	
EURGen-RLC-032 ^c	BACT	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	-				16	0	0	1	
	DNA	x	-	x	x	x	x	-	x	x	x	x	-	-	x	x	-	-	x	x		12	4	2	0	
EURGen-RLC-034	BACT	-	-	-	-	-	-	-	-	-	-	x	x	-	-	-	-	-				2	14	0	0	
	DNA	-	-	-	-	-	-	-	-	-	-	x	x	-	-	-	-	-				2	14	0	0	
EURGen-RLC-036	BACT	x	x	x	x	x	x	-	x	x	x	x	x	x	x	x	x	-	x	x		15	1	2	0	
	DNA	x	x	x	x	x	x	-	x	x	x	x	x	x	x	x	x	-	x	x		15	1	2	0	
EURGen-RLC-012																						NA	NA	NA	NA	
Correct (nr.)		42	40	41	44	44	24	44	44	42	46	46	39	38	42	43	21	NA	NA	NA		Total	663	73	55	21
Missing or UN (nr.)		4	6	5	2	2	2	22	2	2	4	0	0	7	8	4	3	25	26	23	6					

IN SILICO PREDICTION OF AMR PROFILES – SUBMITTED RESULTS

Strain EURGen-2024-04 (*K. pneumoniae*)

- Missing from most submitted results
 - Ceftazidime-avibactam (n=22)
 - Missing from *bla*_{NDM-5} R-profile in ResFinder database
- Reporting intrinsic resistance
 - Ampicillin (n=26)
 - Listed as expected resistance phenotype by EUCAST (intrinsic *bla*SHV)
 - Fosfomycin (n=23)
 - fosA* is intrinsic in *K. pneumoniae*

Results must be evaluated critically:

- Results can / should be confirmed with other tools
- Points to insufficient knowledge of genetic mechanisms of AMR

GENERAL RECOMMENDATIONS

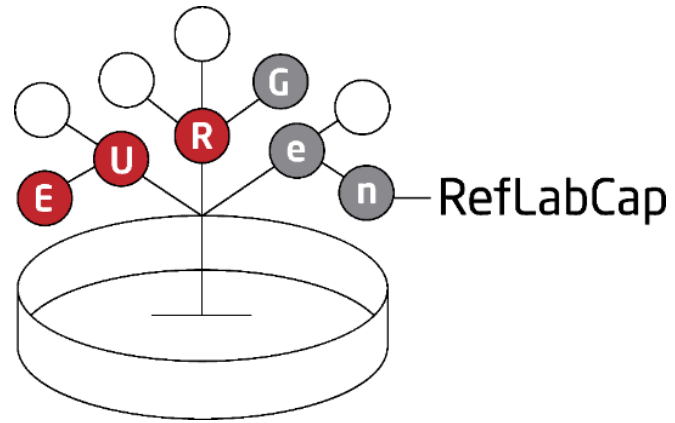
- For discrepancies due to misinterpretation of the EQA protocol and/or insufficient knowledge about certain genetic elements:
 - Laboratories should ensure sufficient knowledge about the genetic mechanisms mediating AMR and other important genetic elements;
 - Laboratories should ensure the use of multiple bioinformatics tools and databases for the detection of genetic determinants since bioinformatics tools and databases can be limited to the analysis of only a few bacterial species which contributes to the false-negative results;
 - Laboratories should be familiar with the bioinformatics tools they use, and the contents of the respective databases

GENERAL RECOMMENDATIONS

- For discrepancies due to variations between the type of data and the chosen bioinformatics tools and databases:
 - Laboratories should implement verification steps such as using multiple bioinformatics approaches to confirm the obtained results;
 - Laboratories should communicate their suggestions, strange observations and potential problems to the curators of bioinformatics tools and databases;
 - Laboratories should be aware of differences between short-and long-read sequencing data and select the most adequate approach depending on their aims.

FEEDBACK SURVEY

- Questions
 - How useful was this EQA to your laboratory? (scale:1-10)
 - Was the preliminary individual EQA evaluation report you received clear and useful? (Yes/No)
 - Did you take any corrective action(s)? (Yes/No)
 - Comment section for suggestions
- Via this link: <https://ec.europa.eu/eusurvey/runner/ba959a0a-2d7a-649f-1719-1a61ace0b253>



Thank you on behalf of the EURGen-RefLabCap team

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