EURGen-RefLabCap

Results from the first external quality assessment (EQA) exercise

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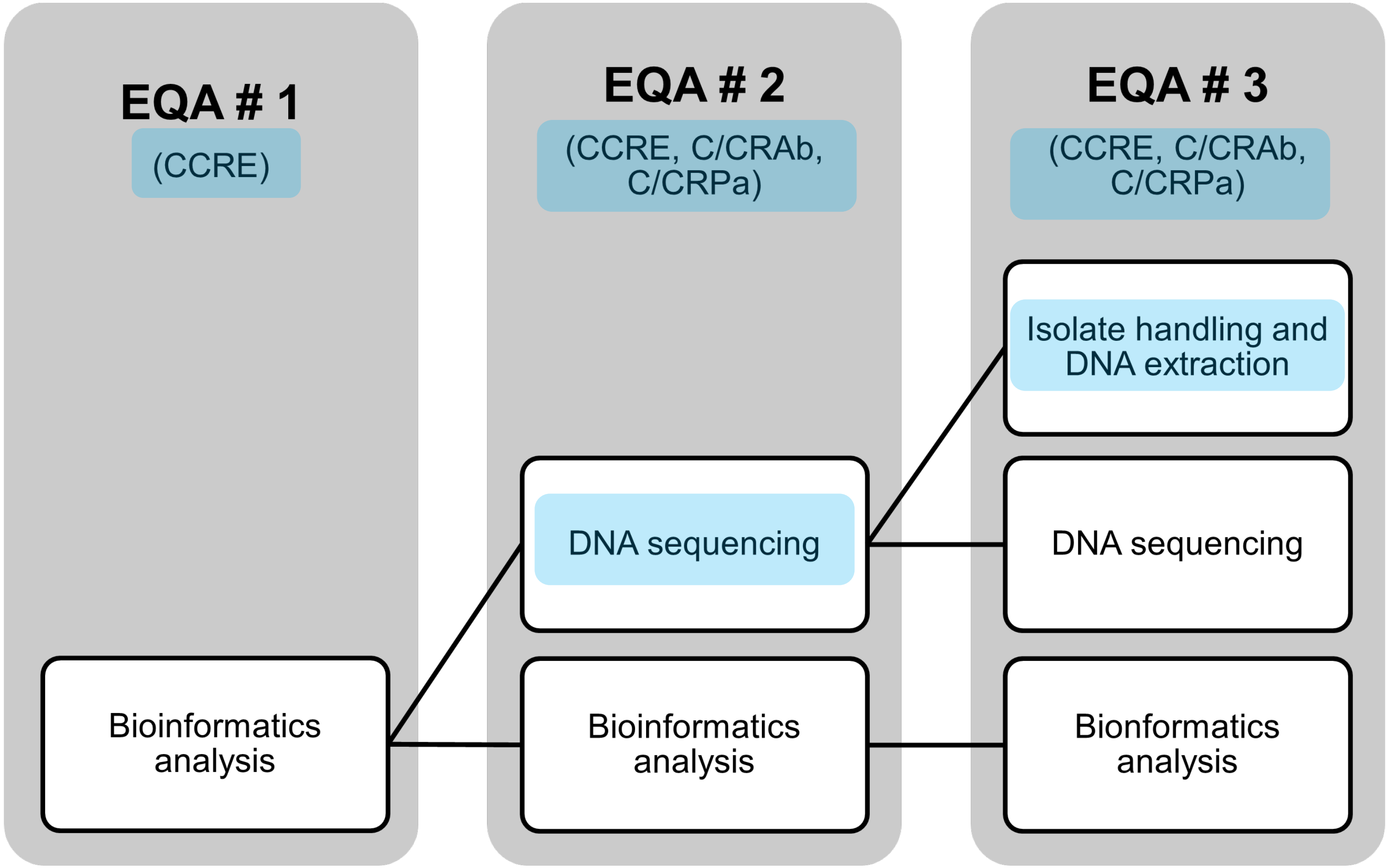
INTRODUCTION

- OVERVIEW OF ALL EURGEN-REFLABCAP EQAS
- DESIGN OF THE FIRST EQA
- PREPARATION OF EXPECTED RESULTS
- SCORING SYSTEM

OVERVIEW OF ALL EURGEN-REFLABCAP EQAS



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DESIGN OF THE FIRST EQA

Materials:

FASTA short-reads

FASTA long-reads

FASTQ short-reads

FASTQ long-reads

Strains:

EURGen-2022-01 *E. coli*

EURGen-2022-02 *K. pneumoniae*

EURGen-2022-03 *K. pneumoniae*

EURGen-2022-04 *E. coli*

Analyses:

i) prediction of multi-locus sequence

ii) detection of plasmid replicon types

iii) detection of genes and chromosomal point mutations mediating AMR

iv) in silico prediction of the AMR profiles

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PREPARATION OF EXPECTED RESULTS

- Three external reference laboratories
  - DTU
  - SSI
  - Centre Hospitalier Universitaire de Caen Normandie
- Mainly tools from Center for Genomic Epidemiology (CGE)
- Default thresholds (80% ID and 60% COV) or higher

Final set of expected results:

Categorical agreement

\+

ID ≥ 90%

\+

COV ≥ 90% (plasmids) / ≥ 60% (ARGs)

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SCORING SYSTEM

Analysis	Submitted result	Score
Prediction of MLST	Correct MLST	**1**
	Incorrect MLST	**0**
Detection of plasmid replicons, AMR genes and chromosomal PMs	Genetic determinant correctly identified	**1**
	Missing a genetic determinant	**blank**
	Reporting a genetic determinant that was not part of the expected results	**0**
In silico prediction of AMR profiles	Complete AMR profile correctly predicted	**1**
	Missing one or more antimicrobial in the complete AMR profile, or including antimicrobials that were not part of the expected profile	**0**

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SCORING SYSTEM

- The *in silico* prediction of AMR profiles was evaluated as a **single answer:**
  - The antimicrobials included in the AMR profiles were not evaluated individually
  - All antimicrobials were evaluated together as one complete AMR profile:
    - To score of “1” the participants had to correctly identify all antimicrobials that were part of the complete AMR profiles
    - Missing one antimicrobial, or including unexpected antimicrobials scored the answer as wrong with a value of “0”

- Example: EURGen-2022-01

Expected	AMC	AMP	CEP	CTA	CTZ	CTV	ERT	IMI	MER	PIT	Unexp.	**Score**
Lab 1	x	x	x	x	x	x	x	x	x	x		**1**
Lab 2	x	-	x	x	x	x	x	x	x	x		**0**
Lab 3	x	x	x	x	x	x	x	x	x	x	(COL)	**0**

SCORING SYSTEM

Maximum possible score for the participants

Material and analysis	EURGen-2022-01	EURGen-2022-02	EURGen-2022-03	EURGen-2022-04	**Total**
Prediction of MLST	1	1	1	1	**4**
Detection of plasmid replicons	2	7[a]	3	5	**17[a]**
Detection of AMR genes and chromosomal PMs	1	14	13	10	**38**
In silico prediction of AMR profiles	1	1	1	1	**4**
Total	**5**	**23[a]**	**18**	**17**	**63[a]**

[a] If using data produced by long-read sequencing, the maximum possible score is *n*+1 due to the presence of one extra expected plasmid replicon in those data, when compared with short-read data

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SCORING SYSTEM

- Scoring system: all unexpected genetic determinants receive score “0”

- During validation of the submitted results: special situations were a score “0” was deemed inappropriate:

    - Reporting the genes *bla*$_{\text{SHV-64}}$ or *bla*$_{\text{SHV-67}}$ instead of the expected genes *bla*$_{\text{SHV-11}}$ or *bla*$_{\text{SHV-12}}$
    - Reporting the genes *bla*$_{\text{SHV-1}}$ or *bla*$_{\text{SHV-106}}$ instead of the expected gene *bla*$_{\text{SHV-28}}$
    - Reporting the gene *aac(3)-IIe* instead of the expected gene *aac(3)-IIa*
    - Reporting the gene *aac(6')-Ib* instead of the expected gene *aac(6')-IIc*
    - Reporting *bla*$_{\text{TEM}}$ variants different from *bla*$_{\text{TEM-1}}$
    - Reporting the plasmid replicon IncHI1B(pNDM-MAR), when using short-read data

- The scoring was **manually adjusted** in these situations and the individual evaluation reports for each laboratory were updated: participants no longer receive a score “0” (which indicates an error), but instead will see a result of “blank” (indicating a discrepancy when compared with the expected results).

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RESULTS AND DISCUSSION

- ANALYSED MATERIALS
- OVERALL SCORES OF THE PARTICIPANTS
- PREDICTION OF MLST
- DETECTION OF PLASMID REPLICONS
- DETECTION OF GENES AND CHROMOSOMAL POINT MUTATIONS MEDIATING AMR
- *IN SILICO* PREDICTION OF AMR PROFILES

ANALYSED MATERIALS

Total short-reads = 28

Total long-reads = 7

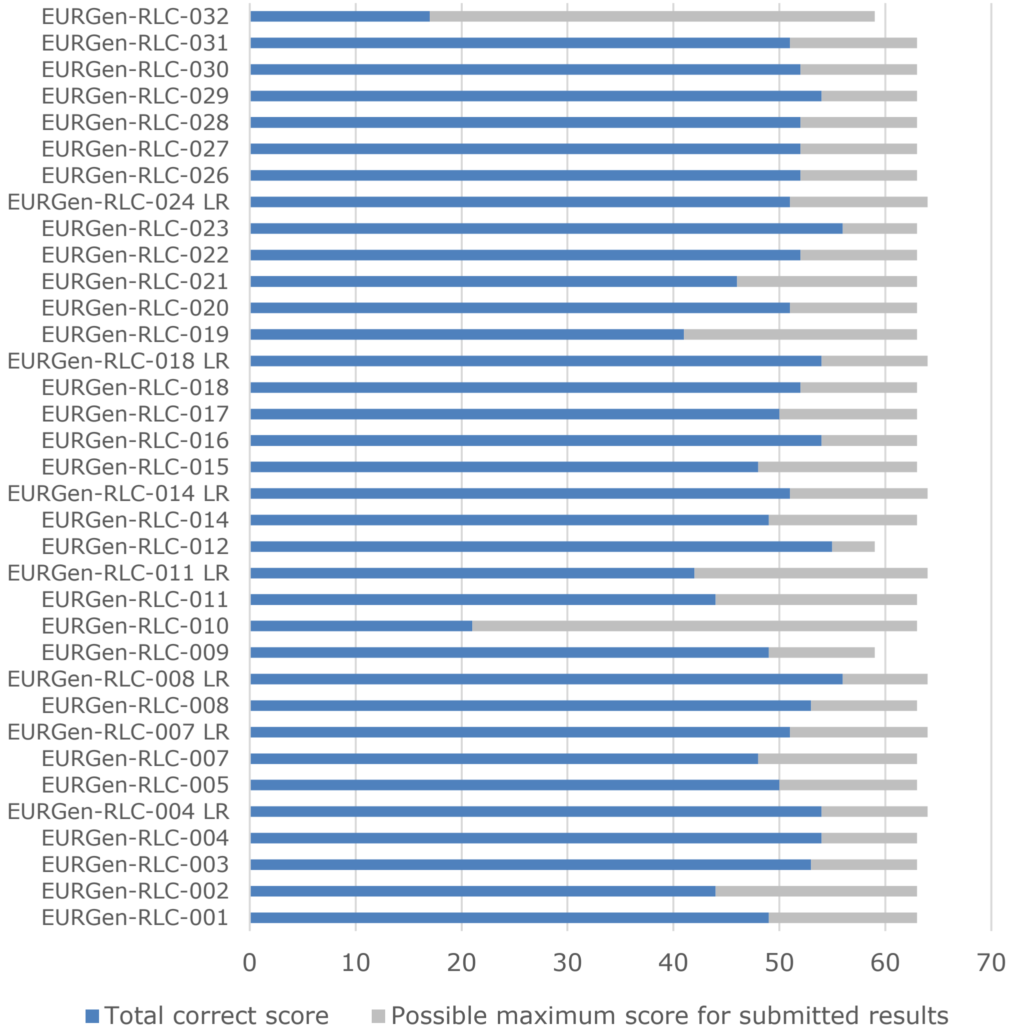
	FASTA short-reads	FASTA long-reads	FASTQ short-reads	FASTQ long-reads
Number of sequence analyses	**18**	**6**	**10**	**1**

Total 24 FASTA	Total 11 FASTQ

Total 35 analyses

Most common bioinformatics approaches: CGE tools

OVERALL SCORES OF THE PARTICIPANTS



<u>Averages of scores (%):</u>

Prediction of MSLT: **96.4%**

Detection of plasmid replicons: **74.0%**

Prediction of genetic AMR determinants: **83.3%**

Prediction of AMR profiles: **14.8% ***

Total: 77.6%

* Analysis of individual AMR profiles: **87.6%**

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PREDICTION OF MLST – EXPECTED RESULTS

Material	**MLST**	**Alleles assigned to each loci, from the scheme *E. coli* #1**						
		adk	fumC	gyrB	icd	mdh	purA	recA
EURGen-2022-01	399	6	4	1	95	69	8	20
EURGen-2022-04	635	6	107	1	95	69	8	7

Material	**MLST**	**Alleles assigned to each loci, from the scheme *K. pneumoniae***						
		gapA	infB	mdh	pgi	phoE	rpoB	tonB
EURGen-2022-02	147[a]	3[a]	4	6	1	7	4	38
EURGen-2022-03	307	4	1	2	52	1	1	7

[a] The assembled file produced with short-read sequencing yielded a perfect hit for the *gapA* locus, but the file produced with long-read sequencing did not generate a perfect hit.

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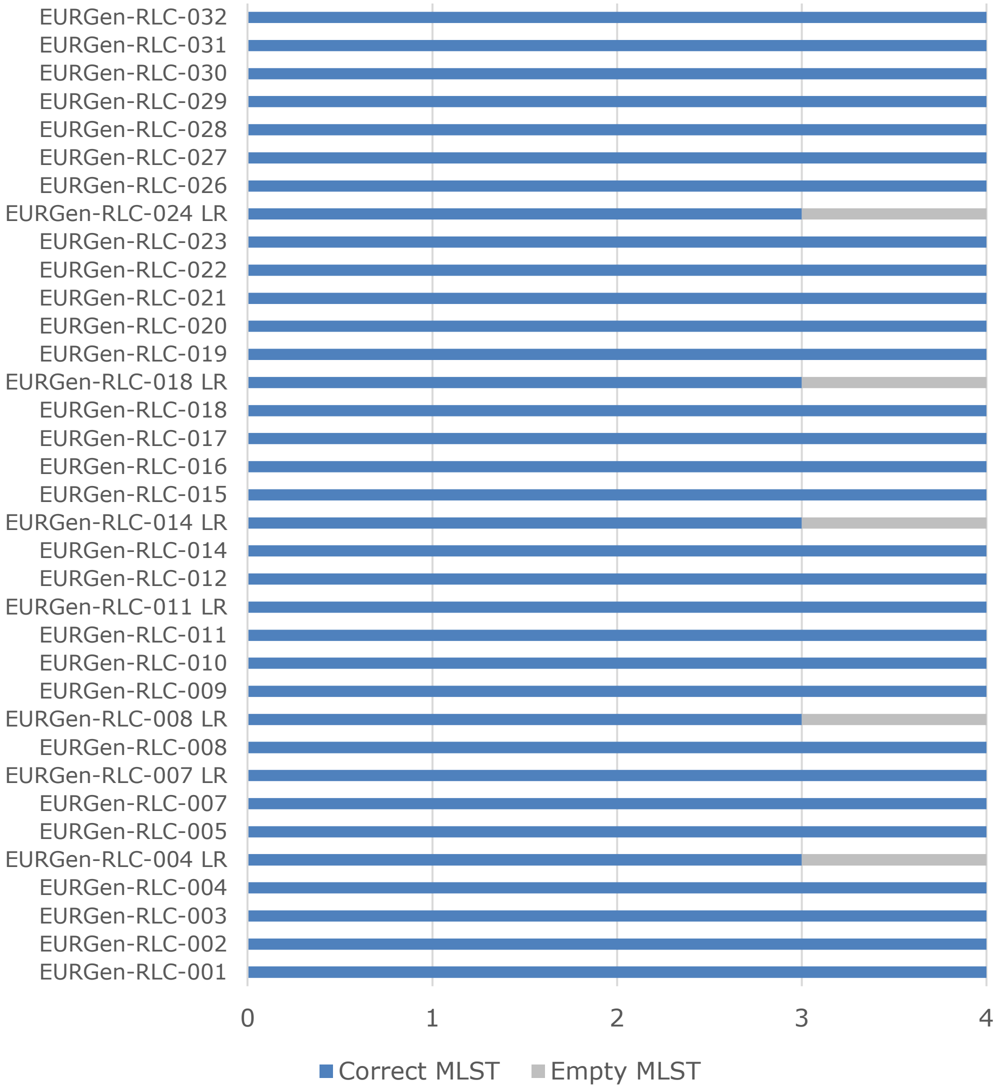
PREDICTION OF MLST – SUBMITTED RESULTS



96.4% correct (n=135)

3.6% empty (n=5)

EURGen-2022-02
Long-reads

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PREDICTION OF MLST – DISCUSSION

The five empty MLST results:

- Absence of a perfect hit for the *gapA* loci
- Also seen in the expected results

Discrepancy between short- and long-read data:

- there are differences between these technologies
- generally, short-read sequencing data have higher accuracy for analyses that are sensitive to SNPs

Recommendations:

- During routine laboratory work report the closest MLST
- Report the imperfect allele to the respective database

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DETECTION OF PLASMID REPLICONS – EXPECTED RESULTS

Material	Plasmid replicons	Nr.
EURGen-2022-01	IncN2; IncFIB(K)	2
EURGen-2022-02	IncFII; IncL; IncFII(Yp); IncFIB(K)(pCAV1099-114); Col156; IncFIB(pB171); Col(pHAD28); IncHI1B(pNDM-MAR)[a]	7 / 8
EURGen-2022-03	IncFIB(pQil); IncFIB(K); IncFII(K)	3
EURGen-2022-04	IncHI2A; IncHI2; IncFII; IncFIB(K); Col(pHAD28)	5

[a] The replicon was only expected in data produced with long-read sequencing technologies. Participants that requested to analyse FASTQ files, including those using files produced through short-read sequencing, could also potentially detect the replicon. However, the replicon was not included nor scored as part of expected results for short-read data since its presence was not uniform between FASTQ and FASTA datasets.

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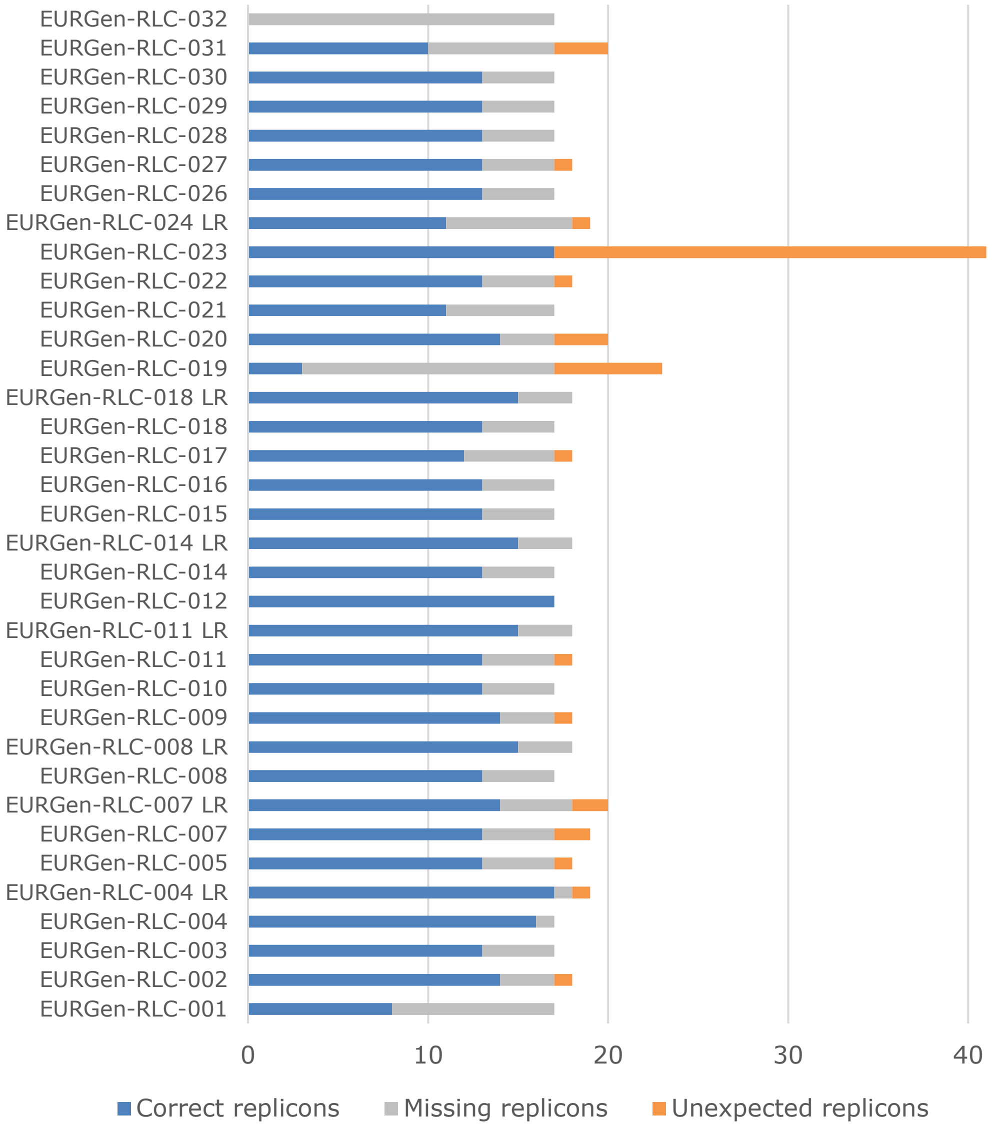
DETECTION OF PLASMID REPLICONS – SUBMITTED RESULTS



48.6% fully correct (n=68)

47.9% missing replicons (n=67)

18.6% with unexpected replicons (n=26)

15% simultaneous (n=21)

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DETECTION OF PLASMID REPLICONS – SUBMITTED RESULTS

Missing replicons in strain 01	Type of data
IncFIB(K) (n=3)	SR
IncN2 (n=2)	SR

Missing replicons in strain 02	Type of data
Col(pHAD28) (n=25)	SR
Col(pHAD28) (n=6)	LR
Col156 (n=22)	SR
Col156 (n=5)	LR
IncFIB(pB171) (n=22)	SR
IncFIB(pB171) (n=5)	LR
IncFIB(K)(pCAV1099-114) (n=7)	SR
IncFIB(K)(pCAV1099-114) (n=2)	LR
IncFII(Yp) (n=5)	SR
IncL (n=2)	SR
IncL (n=2)	LR
IncFII (n=1)	SR
IncFII (n=1)	LR

Missing replicons in strain 03	Type of data
IncFII(K) (n=1)	LR
IncFII(K) (n=5)	SR
IncFIB(K) (n=4)	SR
IncFIB(pQil) (n=2)	SR

Missing replicons in strain 04	Type of data
Col(pHAD28) (n=2)	LR
Col(pHAD28) (n=23)	SR
IncFIB(K) (n=3)	SR
IncFII (n=3)	SR
IncHI2 (n=2)	SR
IncHI2A (n=1)	SR

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DETECTION OF PLASMID REPLICONS – DISCUSSION

The difference between expected results:

- IncHI1B(pNDM-MAR) in strain 02 was only part of the expected results for long-read data

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 (?)
- Other (?)

Different approaches according to purpose:

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

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DETECTION OF PLASMID REPLICONS – DISCUSSION

Reported unexpected results:

- One participant with 24 unexpected replicons

Results must be evaluated critically:

- Too much noise can hide important information
- Results can / should be confirmed with other tools

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DETECTION OF GENES AND MUTATIONS MEDIATING AMR AND PREDICTION OF AMR PROFILES – EXPECTED RESULTS

Material	AMR genes and chromosomal PMs	Nr.	Associated prediction of AMR profiles	Nr.
EURGen-2022-01	bla$_{\text{NDM-1}}$	1	Amoxicillin-clavulanic acid, ampicillin, cefepime, cefotaxime, ceftazidime, ceftazidime-avibactam, ertapenem, imipenem, meropenem, piperacillin-tazobactam	10
EURGen-2022-02	bla$_{\text{CTX-M-15}}$, bla$_{\text{NDM-1}}$, bla$_{\text{OXA-1}}$, bla$_{\text{OXA-48}}$, bla$_{\text{SHV-11}}$, bla$_{\text{SHV-12}}$, bla$_{\text{TEM-1}}$, rmtC, aac(6')-Ib-cr, aac(3)-Iia, aac(6')-Ib-cr, gyrA S83I, parC S80I, dfrA17, sul1	14	Amoxicillin-clavulanic acid, ampicillin, aztreonam, cefepime, cefotaxime, ceftazidime, ceftazidime-avibactam, ertapenem, imipenem, meropenem, piperacillin-tazobactam, Amikacin, gentamicin, tobramycin, Ciprofloxacin, Trimethoprim, Sulfamethoxazole	17
EURGen-2022-03	bla$_{\text{KPC-3}}$, bla$_{\text{CTX-M-15}}$, bla$_{\text{OXA-1}}$, bla$_{\text{OXA-9}}$, bla$_{\text{TEM-1}}$, bla$_{\text{SHV-28,}}$ aac(3)-IIa, aac(6')-Ib-cr, qnrB1, aac(6')-Ib-cr, gyrA S83I, parC S80I, dfrA14, sul2, mgrB::IS1	13 (+1)	Amoxicillin-clavulanic acid, ampicillin, aztreonam, cefepime, cefotaxime, ceftazidime, ertapenem, imipenem, meropenem, piperacillin-tazobactam, Amikacin, gentamicin, tobramycin, Ciprofloxacin, Trimethoprim, Sulfamethoxazole, Colistin	16 (+1)
EURGen-2022-04	bla$_{\text{OXA-10}}$, bla$_{\text{OXA-436}}$, bla$_{\text{SHV-12}}$, bla$_{\text{TEM-1}}$, aac(6')-IIc, gyrA S83I, qnrA1, dfrA19, sul1, sul2	10	Amoxicillin-clavulanic acid, ampicillin, aztreonam, cefepime, cefotaxime, ceftazidime, ertapenem, imipenem, meropenem, piperacillin-tazobactam, Gentamicin, tobramycin, Ciprofloxacin, Trimethoprim, Sulfamethoxazole	15

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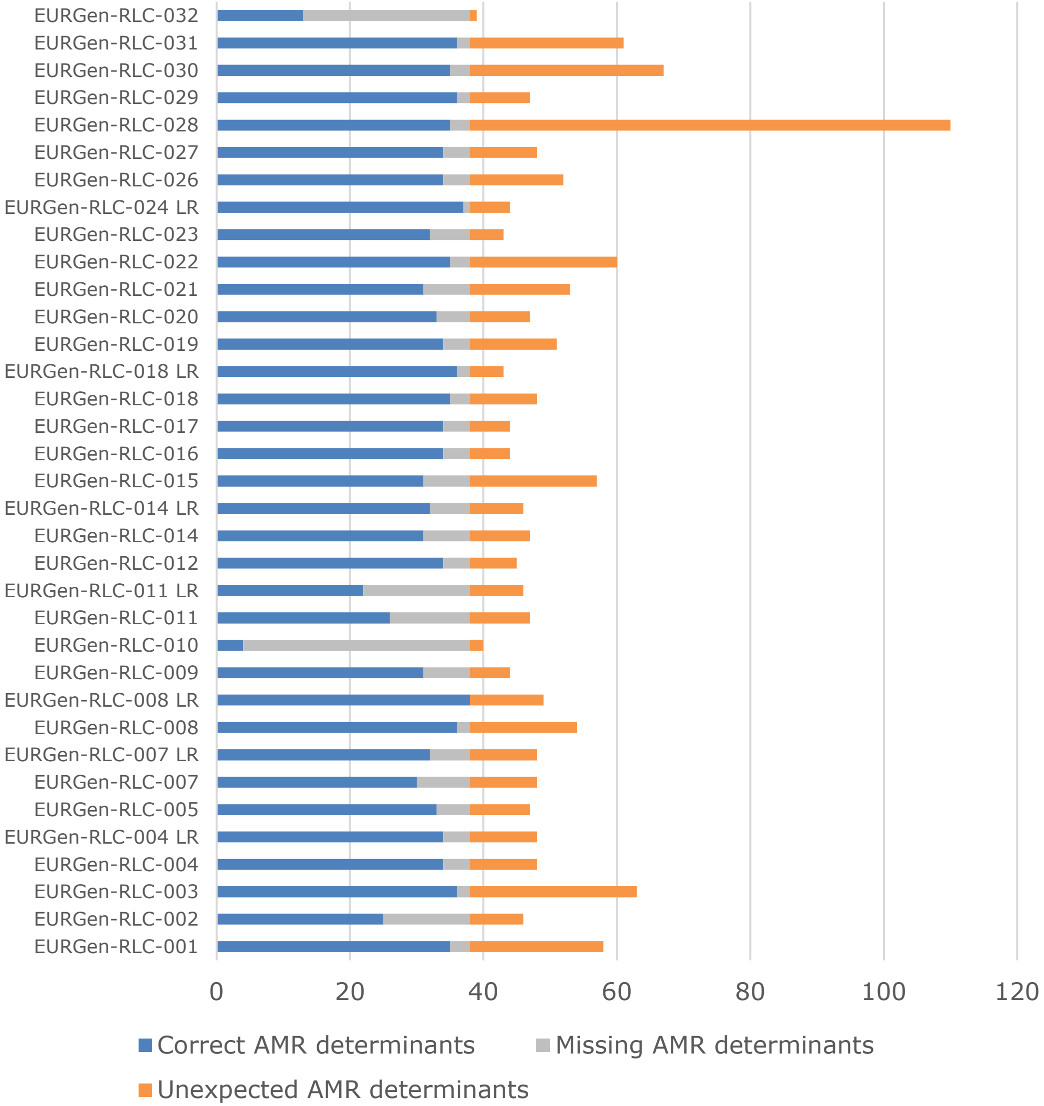
DETECTION OF GENES AND MUTATIONS – SUBMITTED RESULTS



24.3% fully correct (n=34)

58.6% missing determinants (n=82)

72.1% with unexpected determinants (n=26)

55% simultaneous (n=77)

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DETECTION OF GENES AND MUTATIONS – SUBMITTED RESULTS

Strain	**Total expected**	**Correct**	**Missing**	**Unexpected**
01	35	35 (100%)	0	7 (↑ 20%)
02	490	405 (82.7%)	85 (17.3%)	176 (↑ 36%)
03	455	369 (81.1%)	86 (18.9%)	224 (↑ 49%)
04	350	299 (85.4%)	51 (14.6%)	45 (↑ 13%)

Strain	**Examples of problems**
02	Missing blaSHV-11 (n=25)
	Missing gyrA or parC PM (n=18)
	Missing aac(3)-IIa (n=15)
	Unexpected fosA (n=22)
	Unexpected oqxA/oqxB (n=20)
	Unexpected aac(3)-IIe (n=10)
	Unexpected PM in acrR (n=58)
	Unexpected PM in ramR (n=11)
03	Unexpected blaTEM (n=47)
04	Unexpected mcr-9 (n=31)

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DETECTION OF GENES AND MUTATIONS – DISCUSSION

Strain	Examples of problems
02	Missing blaSHV-11 (n=25)
	Missing gyrA or parC PM (n=18)
	Missing aac(3)-IIa (n=15)
	Unexpected fosA (n=22)
	Unexpected oqxA/oqxB (n=20)
	Unexpected aac(3)-IIe (n=10)
	Unexpected PM in acrR (n=58)
	Unexpected PM in ramR (n=11)
03	Unexpected blaTEM (n=47)
04	Unexpected mcr-9 (n=31)

Several variants from the same gene present simultaneously:

- Participants failed to report one of the variants

⇩

Results must be evaluated critically:

- The different variants are not in the same genomic location and are present simultaneously

Different approaches according to purpose:

- Results can / should be confirmed with other tools

DETECTION OF GENES AND MUTATIONS – DISCUSSION

Strain	Examples of problems
02	Missing blaSHV-11 (n=25)
	Missing gyrA or parC PM (n=18)
	Missing aac(3)-IIa (n=15)
	Unexpected fosA (n=22)
	Unexpected oqxA/oqxB (n=20)
	Unexpected aac(3)-IIe (n=10)
	Unexpected PM in acrR (n=58)
	Unexpected PM in ramR (n=11)
03	Unexpected blaTEM (n=47)
04	Unexpected mcr-9 (n=31)

Reporting an incomplete PM profile:

- ○ Impact on expected phenotype (cumulative effect)

Results must be evaluated critically:

- Results can / should be confirmed with other tools

DETECTION OF GENES AND MUTATIONS – DISCUSSION

Strain	Examples of problems
02	Missing blaSHV-11 (n=25)
	Missing gyrA or parC PM (n=18)
	Missing aac(3)-IIa (n=15)
	Unexpected fosA (n=22)
	Unexpected oqxA/oqxB (n=20)
	Unexpected aac(3)-IIe (n=10)
	Unexpected PM in acrR (n=58)
	Unexpected PM in ramR (n=11)
03	Unexpected blaTEM (n=47)
04	Unexpected mcr-9 (n=31)

Differences between bioinformatics databases:

- The same genetic sequence is annotated differently in ResFinder and AMRFinderPlus

⇩

Results must be evaluated critically:

- Important to understand the different databases supporting the tools
- Results can / should be confirmed with other tools

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DETECTION OF GENES AND MUTATIONS – DISCUSSION

Strain	Examples of problems
02	Missing blaSHV-11 (n=25)
	Missing gyrA or parC PM (n=18)
	Missing aac(3)-IIa (n=15)
	Unexpected fosA (n=22)
	Unexpected oqxA/oqxB (n=20)
	Unexpected aac(3)-IIe (n=10)
	Unexpected PM in acrR (n=58)
	Unexpected PM in ramR (n=11)
03	Unexpected blaTEM (n=47)
04	Unexpected mcr-9 (n=31)

Reporting intrinsic genes:

○ Present in strain but with no impact in WT vs. non-WT phenotypes

Results must be evaluated critically:

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

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DETECTION OF GENES AND MUTATIONS – DISCUSSION

Strain	Examples of problems
02	Missing blaSHV-11 (n=25)
	Missing gyrA or parC PM (n=18)
	Missing aac(3)-IIa (n=15)
	Unexpected fosA (n=22)
	Unexpected oqxA/oqxB (n=20)
	Unexpected aac(3)-IIe (n=10)
	Unexpected PM in acrR (n=58)
	Unexpected PM in ramR (n=11)
03	Unexpected blaTEM (n=47)
04	Unexpected mcr-9 (n=31)

Reporting PM (or genes) not relevant for the EQA

- ○ Including mutations without proved association with R-profiles

⇩

<u>Results must be evaluated critically:</u>

- Too much noise can hide important information
- Might point to insufficient knowledge of genetic AMR mechanisms

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DETECTION OF GENES AND MUTATIONS – DISCUSSION

Strain	Examples of problems
02	Missing blaSHV-11 (n=25)
	Missing gyrA or parC PM (n=18)
	Missing aac(3)-IIa (n=15)
	Unexpected fosA (n=22)
	Unexpected oqxA/oqxB (n=20)
	Unexpected aac(3)-IIe (n=10)
	Unexpected PM in acrR (n=58)
	Unexpected PM in ramR (n=11)
03	Unexpected blaTEM (n=47)
04	Unexpected mcr-9 (n=31)

Reported unexpected results:

- Nine participants reporting >2 variants of blaTEM from the same genomic location

<u>Results must be evaluated critically:</u>

- Too much noise can hide important information
- Results can / should be confirmed with other tools
- Different genetic variants can have different phenotypic implications

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DETECTION OF GENES AND MUTATIONS – DISCUSSION

Strain	Examples of problems
02	Missing blaSHV-11 (n=25)
	Missing gyrA or parC PM (n=18)
	Missing aac(3)-IIa (n=15)
	Unexpected fosA (n=22)
	Unexpected oqxA/oqxB (n=20)
	Unexpected aac(3)-IIe (n=10)
	Unexpected PM in acrR (n=58)
	Unexpected PM in ramR (n=11)
03	Unexpected blaTEM (n=47)
04	Unexpected mcr-9 (n=31)

Reporting different variants that occupy the same position in the genome:

- Present in the strain, but does not confer phenotypic resistance

Results must be evaluated critically:

- Too much noise can hide important information
- Points to insufficient knowledge of genetic AMR mechanisms
- Might lead to incorrect reporting of R-profiles

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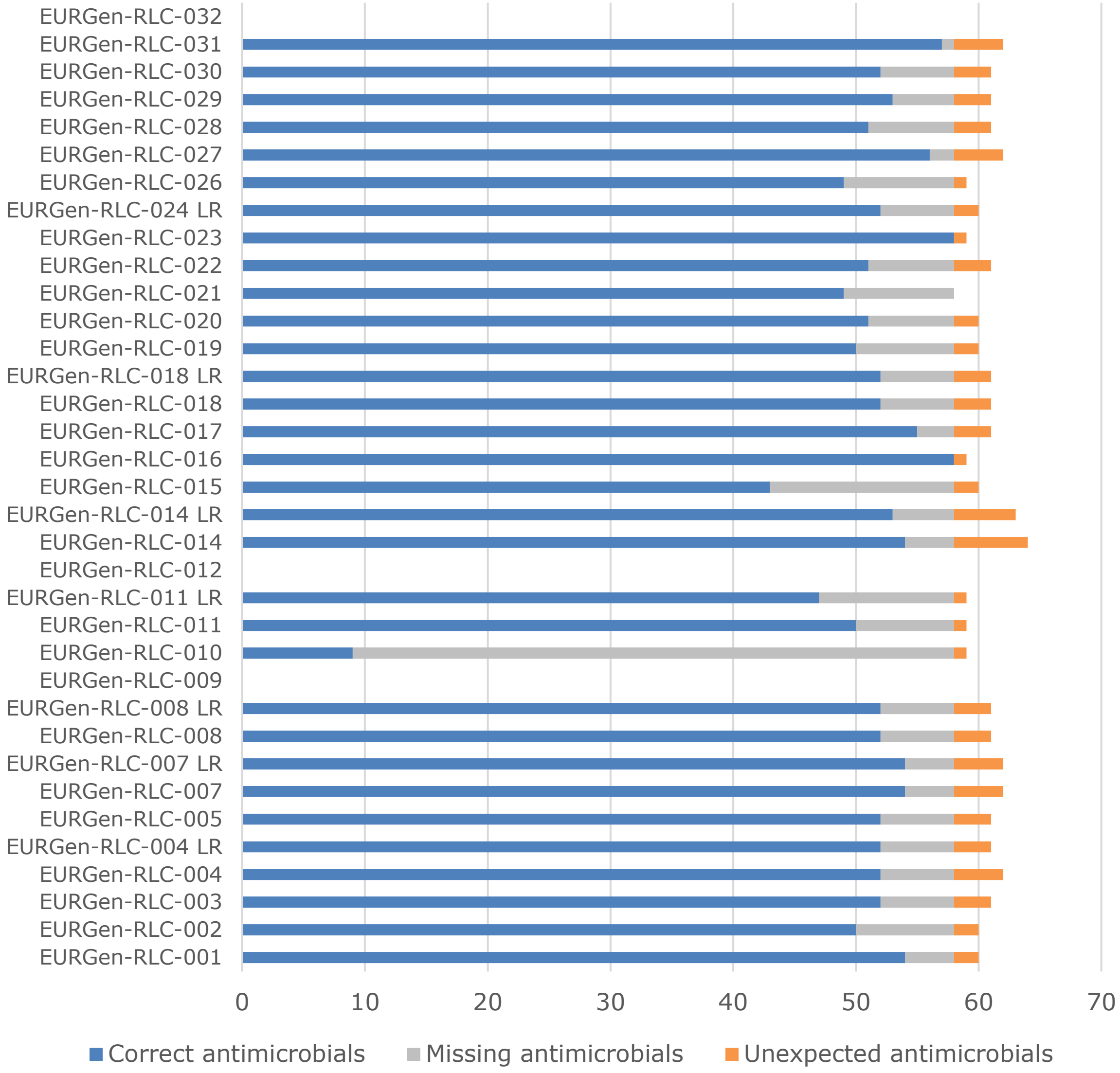
IN SILICO PREDICTION OF AMR PROFILES – MANUAL ANALYSIS



14.8% fully correct (n=19)

63.3% missing antimicrobials (n=81)

60.9% with unexpected antimicrobials (n=78)

39.1% simultaneous (n=50)

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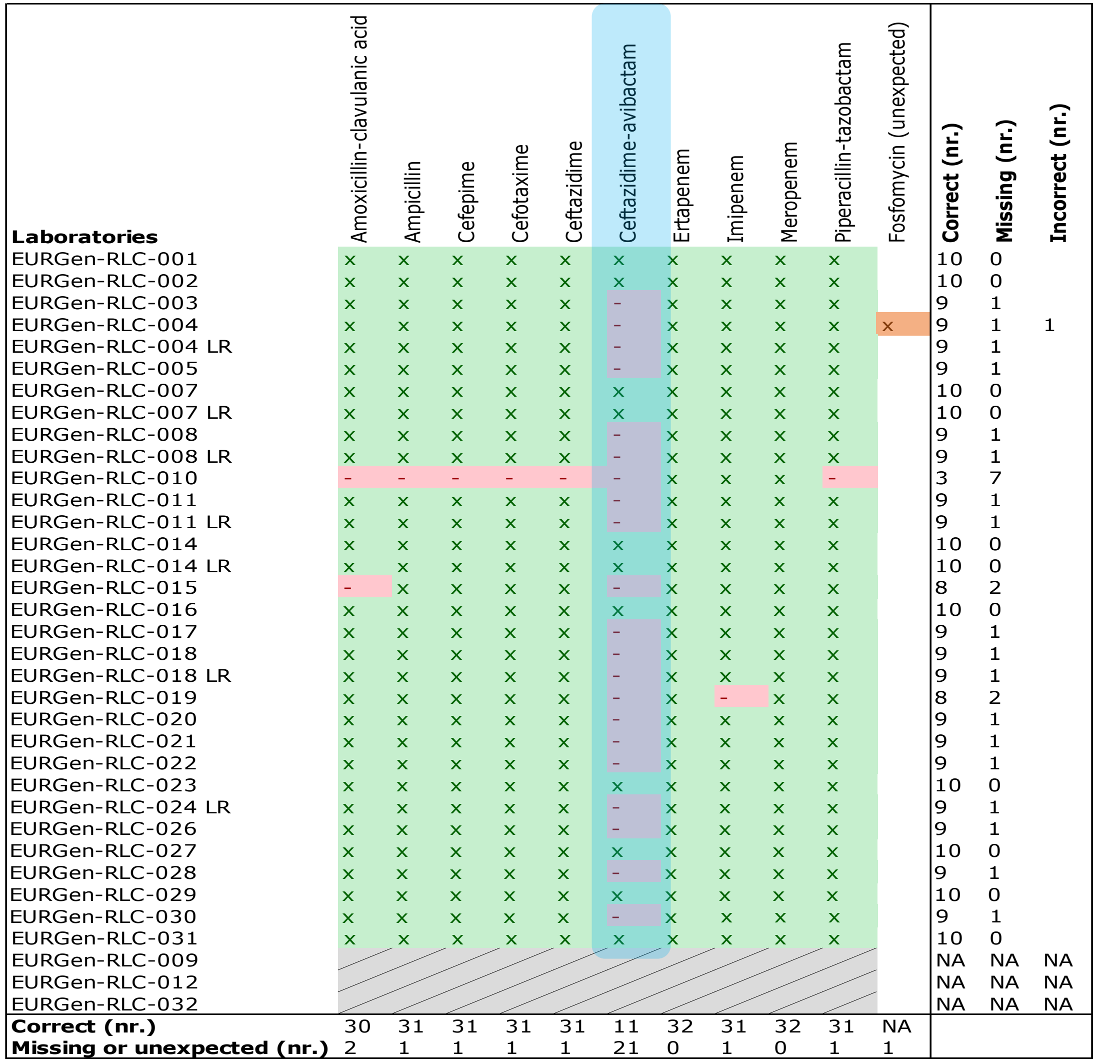
IN SILICO PREDICTION OF AMR PROFILES – MANUAL ANALYSIS

Strain 01 E. coli



Laboratories	Amoxicillin-clavulanic acid	Ampicillin	Cefepime	Cefotaxime	Ceftazidime	Ceftazidime-avibactam	Ertapenem	Imipenem	Meropenem	Piperacillin-tazobactam	Fosfomycin (unexpected)	Correct (nr.)	Missing (nr.)	Incorrect (nr.)
EURGen-RLC-001	x	x	x	x	x	x	x	x	x	x		10	0	
EURGen-RLC-002	x	x	x	x	x	x	x	x	x	x		10	0	
EURGen-RLC-003	x	x	x	x	x	-	x	x	x	x		9	1	
EURGen-RLC-004	x	x	x	x	x	-	x	x	x	x	x	9	1	1
EURGen-RLC-004 LR	x	x	x	x	x	-	x	x	x	x		9	1	
EURGen-RLC-005	x	x	x	x	x	-	x	x	x	x		9	1	
EURGen-RLC-007	x	x	x	x	x	x	x	x	x	x		10	0	
EURGen-RLC-007 LR	x	x	x	x	x	x	x	x	x	x		10	0	
EURGen-RLC-008	x	x	x	x	x	-	x	x	x	x		9	1	
EURGen-RLC-008 LR	x	x	x	x	x	-	x	x	x	x		9	1	
EURGen-RLC-010	-	-	-	-	-	-	x	x	x	-		3	7	
EURGen-RLC-011	x	x	x	x	x	-	x	x	x	x		9	1	
EURGen-RLC-011 LR	x	x	x	x	x	-	x	x	x	x		9	1	
EURGen-RLC-014	x	x	x	x	x	x	x	x	x	x		10	0	
EURGen-RLC-014 LR	x	x	x	x	x	x	x	x	x	x		10	0	
EURGen-RLC-015	-	x	x	x	x	-	x	x	x	x		8	2	
EURGen-RLC-016	x	x	x	x	x	x	x	x	x	x		10	0	
EURGen-RLC-017	x	x	x	x	x	-	x	x	x	x		9	1	
EURGen-RLC-018	x	x	x	x	x	-	x	x	x	x		9	1	
EURGen-RLC-018 LR	x	x	x	x	x	-	x	x	x	x		9	1	
EURGen-RLC-019	x	x	x	x	x	-	x	-	x	x		8	2	
EURGen-RLC-020	x	x	x	x	x	-	x	x	x	x		9	1	
EURGen-RLC-021	x	x	x	x	x	-	x	x	x	x		9	1	
EURGen-RLC-022	x	x	x	x	x	-	x	x	x	x		9	1	
EURGen-RLC-023	x	x	x	x	x	x	x	x	x	x		10	0	
EURGen-RLC-024 LR	x	x	x	x	x	-	x	x	x	x		9	1	
EURGen-RLC-026	x	x	x	x	x	-	x	x	x	x		9	1	
EURGen-RLC-027	x	x	x	x	x	x	x	x	x	x		10	0	
EURGen-RLC-028	x	x	x	x	x	-	x	x	x	x		9	1	
EURGen-RLC-029	x	x	x	x	x	x	x	x	x	x		10	0	
EURGen-RLC-030	x	x	x	x	x	-	x	x	x	x		9	1	
EURGen-RLC-031	x	x	x	x	x	x	x	x	x	x		10	0	
EURGen-RLC-009	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨		NA	NA	NA
EURGen-RLC-012	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨		NA	NA	NA
EURGen-RLC-032	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨		NA	NA	NA
Correct (nr.)	30	31	31	31	31	11	32	31	32	31	NA			
Missing or unexpected (nr.)	2	1	1	1	1	21	0	1	0	1	1			

Missing from most submitted results:

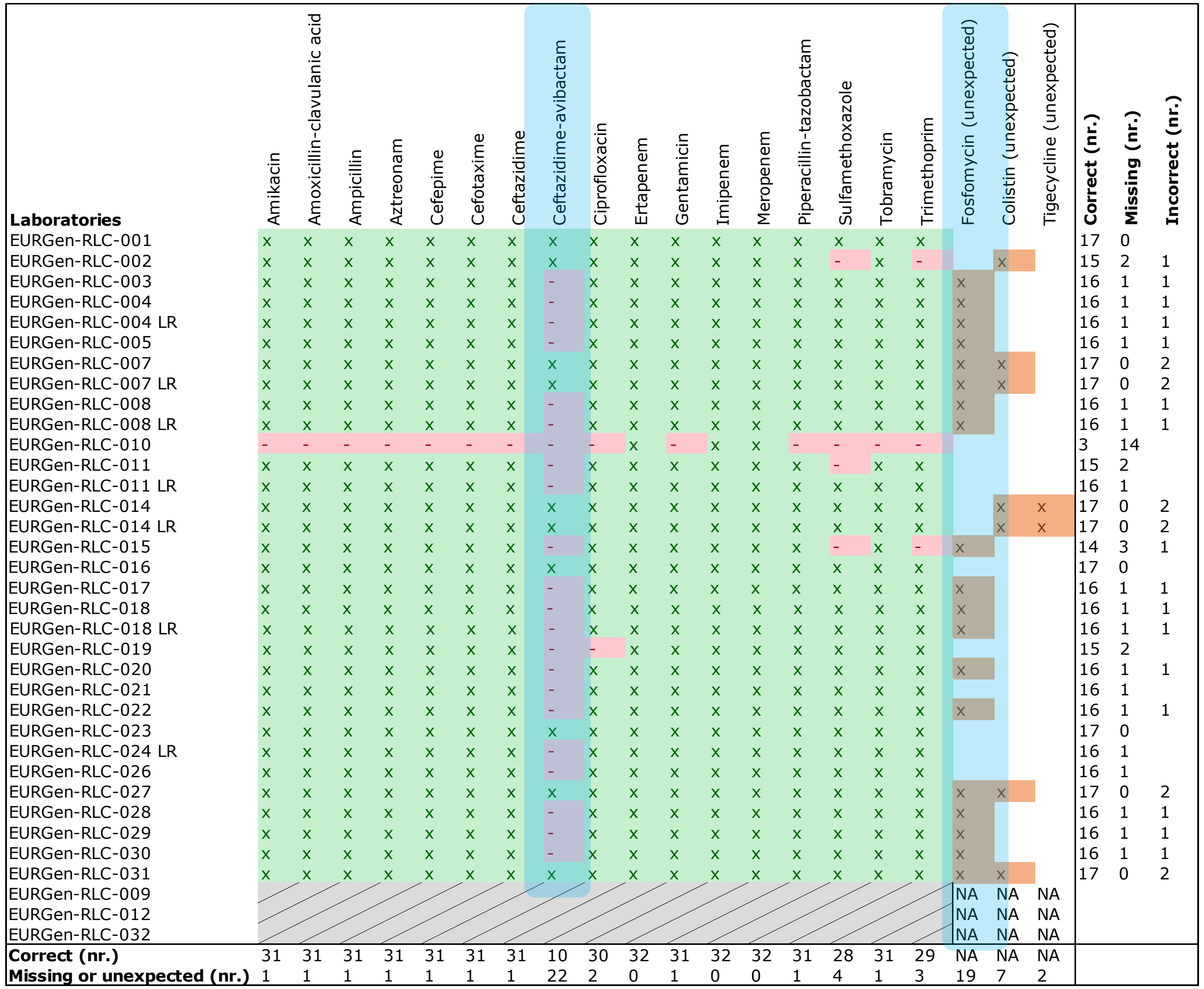
○ Antimicrobial missing from blaNDM-1 R-profile in ResFinder database (but present for other genes)

<u>Results must be evaluated critically:</u>

- Results can / should be confirmed with other tools
- Points to insufficient knowledge of genetic AMR mechanisms
- Important to understand the different databases supporting the tools

IN SILICO PREDICTION OF AMR PROFILES – MANUAL ANALYSIS

Strain 02 K. pneumoniae



Laboratories	Amikacin	Amoxicillin-clavulanic acid	Ampicillin	Aztreonam	Cefepime	Cefotaxime	Ceftazidime	Ceftazidime-avibactam	Ciprofloxacin	Ertapenem	Gentamicin	Imipenem	Meropenem	Piperacillin-tazobactam	Sulfamethoxazole	Tobramycin	Trimethoprim	Fosfomycin (unexpected)	Colistin (unexpected)	Tigecycline (unexpected)	Correct (nr.)	Missing (nr.)	Incorrect (nr.)
EURGen-RLC-001	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x				17	0	
EURGen-RLC-002	x	x	x	x	x	x	x	x	x	x	x	x	x	x	-	x	-		x		15	2	1
EURGen-RLC-003	x	x	x	x	x	x	x	-	x	x	x	x	x	x	x	x	x	x			16	1	1
EURGen-RLC-004	x	x	x	x	x	x	x	-	x	x	x	x	x	x	x	x	x	x			16	1	1
EURGen-RLC-004 LR	x	x	x	x	x	x	x	-	x	x	x	x	x	x	x	x	x	x			16	1	1
EURGen-RLC-005	x	x	x	x	x	x	x	-	x	x	x	x	x	x	x	x	x	x			16	1	1
EURGen-RLC-007	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x		17	0	2
EURGen-RLC-007 LR	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x		17	0	2
EURGen-RLC-008	x	x	x	x	x	x	x	-	x	x	x	x	x	x	x	x	x	x			16	1	1
EURGen-RLC-008 LR	x	x	x	x	x	x	x	-	x	x	x	x	x	x	x	x	x	x			16	1	1
EURGen-RLC-010	-	-	-	-	-	-	-	-	-	x	-	x	x	-	-	-	-				3	14	
EURGen-RLC-011	x	x	x	x	x	x	x	-	x	x	x	x	x	x	-	x	x				15	2	
EURGen-RLC-011 LR	x	x	x	x	x	x	x	-	x	x	x	x	x	x	x	x	x				16	1	
EURGen-RLC-014	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x		x	x	17	0	2
EURGen-RLC-014 LR	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x		x	x	17	0	2
EURGen-RLC-015	x	x	x	x	x	x	x	-	x	x	x	x	x	x	-	x	-	x			14	3	1
EURGen-RLC-016	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x				17	0	
EURGen-RLC-017	x	x	x	x	x	x	x	-	x	x	x	x	x	x	x	x	x	x			16	1	1
EURGen-RLC-018	x	x	x	x	x	x	x	-	x	x	x	x	x	x	x	x	x	x			16	1	1
EURGen-RLC-018 LR	x	x	x	x	x	x	x	-	x	x	x	x	x	x	x	x	x	x			16	1	1
EURGen-RLC-019	x	x	x	x	x	x	x	-	-	x	x	x	x	x	x	x	x				15	2	
EURGen-RLC-020	x	x	x	x	x	x	x	-	x	x	x	x	x	x	x	x	x	x			16	1	1
EURGen-RLC-021	x	x	x	x	x	x	x	-	x	x	x	x	x	x	x	x	x				16	1	
EURGen-RLC-022	x	x	x	x	x	x	x	-	x	x	x	x	x	x	x	x	x	x			16	1	1
EURGen-RLC-023	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x				17	0	
EURGen-RLC-024 LR	x	x	x	x	x	x	x	-	x	x	x	x	x	x	x	x	x				16	1	
EURGen-RLC-026	x	x	x	x	x	x	x	-	x	x	x	x	x	x	x	x	x				16	1	
EURGen-RLC-027	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x		17	0	2
EURGen-RLC-028	x	x	x	x	x	x	x	-	x	x	x	x	x	x	x	x	x	x			16	1	1
EURGen-RLC-029	x	x	x	x	x	x	x	-	x	x	x	x	x	x	x	x	x	x			16	1	1
EURGen-RLC-030	x	x	x	x	x	x	x	-	x	x	x	x	x	x	x	x	x	x			16	1	1
EURGen-RLC-031	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x		17	0	2
EURGen-RLC-009																		NA	NA	NA			
EURGen-RLC-012																		NA	NA	NA			
EURGen-RLC-032																		NA	NA	NA			
Correct (nr.)	31	31	31	31	31	31	31	10	30	32	31	32	32	31	28	31	29	NA	NA	NA			
Missing or unexpected (nr.)	1	1	1	1	1	1	1	22	2	0	1	0	0	1	4	1	3	19	7	2			

Reporting unexpected results:

- ○ Due to do the reporting of intrinsic genes

Results must be evaluated critically:

- Too much noise can hide important information
- Points to insufficient knowledge of genetic AMR mechanisms
- Might lead to incorrect reporting of R-profiles

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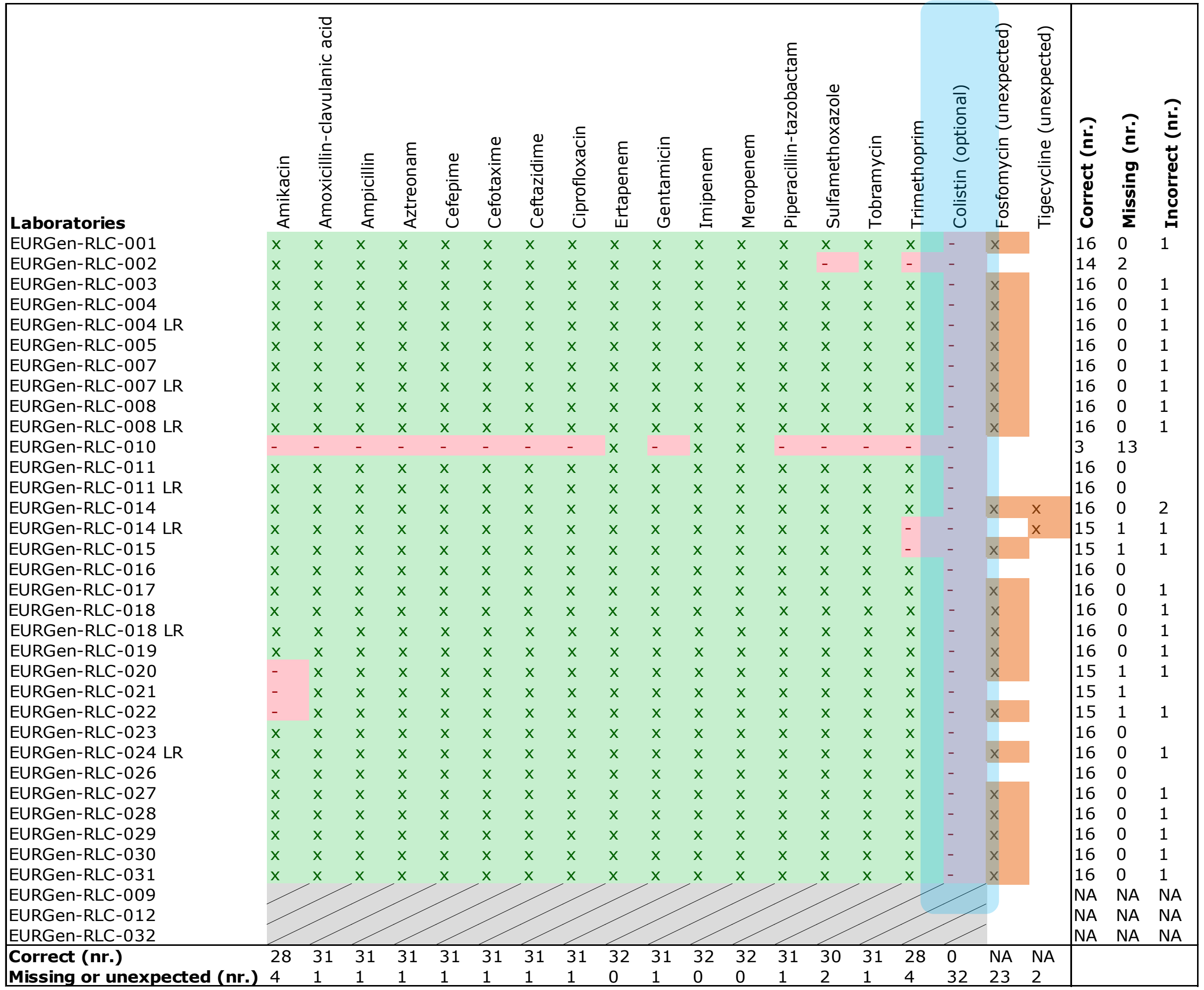
IN SILICO PREDICTION OF AMR PROFILES – MANUAL ANALYSIS

Strain 03 K. pneumoniae



Laboratories	Amikacin	Amoxicillin-clavulanic acid	Ampicillin	Aztreonam	Cefepime	Cefotaxime	Ceftazidime	Ciprofloxacin	Ertapenem	Gentamicin	Imipenem	Meropenem	Piperacillin-tazobactam	Sulfamethoxazole	Tobramycin	Trimethoprim	Colistin (optional)	Fosfomycin (unexpected)	Tigecycline (unexpected)	**Correct (nr.)**	**Missing (nr.)**	**Incorrect (nr.)**
EURGen-RLC-001	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	-	x		16	0	1
EURGen-RLC-002	x	x	x	x	x	x	x	x	x	x	x	x	x	-	x	-	-			14	2	
EURGen-RLC-003	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	-	x		16	0	1
EURGen-RLC-004	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	-	x		16	0	1
EURGen-RLC-004 LR	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	-	x		16	0	1
EURGen-RLC-005	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	-	x		16	0	1
EURGen-RLC-007	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	-	x		16	0	1
EURGen-RLC-007 LR	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	-	x		16	0	1
EURGen-RLC-008	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	-	x		16	0	1
EURGen-RLC-008 LR	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	-	x		16	0	1
EURGen-RLC-010	-	-	-	-	-	-	-	-	x	-	x	x	-	-	-	-	-			3	13	
EURGen-RLC-011	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	-			16	0	
EURGen-RLC-011 LR	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	-			16	0	
EURGen-RLC-014	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	-	x	x	16	0	2
EURGen-RLC-014 LR	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	-	-		x	15	1	1
EURGen-RLC-015	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	-	-	x		15	1	1
EURGen-RLC-016	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	-			16	0	
EURGen-RLC-017	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	-	x		16	0	1
EURGen-RLC-018	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	-	x		16	0	1
EURGen-RLC-018 LR	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	-	x		16	0	1
EURGen-RLC-019	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	-	x		16	0	1
EURGen-RLC-020	-	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	-	x		15	1	1
EURGen-RLC-021	-	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	-			15	1	
EURGen-RLC-022	-	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	-	x		15	1	1
EURGen-RLC-023	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	-			16	0	
EURGen-RLC-024 LR	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	-	x		16	0	1
EURGen-RLC-026	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	-			16	0	
EURGen-RLC-027	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	-	x		16	0	1
EURGen-RLC-028	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	-	x		16	0	1
EURGen-RLC-029	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	-	x		16	0	1
EURGen-RLC-030	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	-	x		16	0	1
EURGen-RLC-031	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	-	x		16	0	1
EURGen-RLC-009																				NA	NA	NA
EURGen-RLC-012																				NA	NA	NA
EURGen-RLC-032																				NA	NA	NA
Correct (nr.)	28	31	31	31	31	31	31	31	32	31	32	32	31	30	31	28	0	NA	NA			
Missing or unexpected (nr.)	4	1	1	1	1	1	1	1	0	1	0	0	1	2	1	4	32	23	2			

None of the participants reported colistin resistance

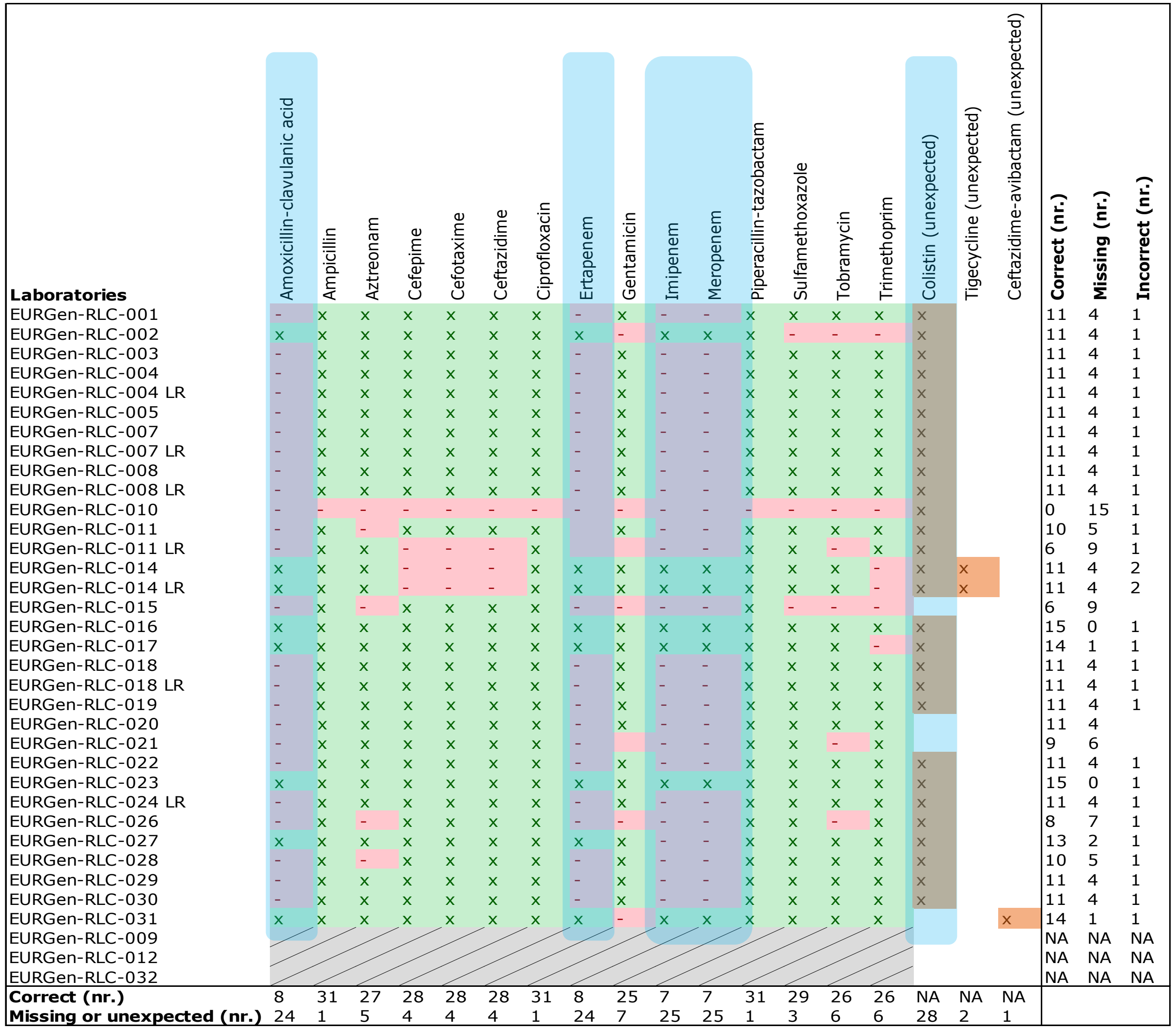
- ○ Because none of the participants found the underlying genetic mechanism of resistance (chromosomal PM mgrB::IS1)

<u>Results must be evaluated critically:</u>

- Results can / should be confirmed with other tools
- Very Major Errors can have important clinical implications

IN SILICO PREDICTION OF AMR PROFILES – MANUAL ANALYSIS

Strain 04 E. coli



Laboratories	Amoxicillin-clavulanic acid	Ampicillin	Aztreonam	Cefepime	Cefotaxime	Ceftazidime	Ciprofloxacin	Ertapenem	Gentamicin	Imipenem	Meropenem	Piperacillin-tazobactam	Sulfamethoxazole	Tobramycin	Trimethoprim	Colistin (unexpected)	Tigecycline (unexpected)	Ceftazidime-avibactam (unexpected)	Correct (nr.)	Missing (nr.)	Incorrect (nr.)
EURGen-RLC-001	-	x	x	x	x	x	x	-	x	-	-	x	x	x	x	x			11	4	1
EURGen-RLC-002	x	x	x	x	x	x	x	x	-	x	x	x	-	-	-	x			11	4	1
EURGen-RLC-003	-	x	x	x	x	x	x	-	x	-	-	x	x	x	x	x			11	4	1
EURGen-RLC-004	-	x	x	x	x	x	x	-	x	-	-	x	x	x	x	x			11	4	1
EURGen-RLC-004 LR	-	x	x	x	x	x	x	-	x	-	-	x	x	x	x	x			11	4	1
EURGen-RLC-005	-	x	x	x	x	x	x	-	x	-	-	x	x	x	x	x			11	4	1
EURGen-RLC-007	-	x	x	x	x	x	x	-	x	-	-	x	x	x	x	x			11	4	1
EURGen-RLC-007 LR	-	x	x	x	x	x	x	-	x	-	-	x	x	x	x	x			11	4	1
EURGen-RLC-008	-	x	x	x	x	x	x	-	x	-	-	x	x	x	x	x			11	4	1
EURGen-RLC-008 LR	-	x	x	x	x	x	x	-	x	-	-	x	x	x	x	x			11	4	1
EURGen-RLC-010	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x			0	15	1
EURGen-RLC-011	-	x	-	x	x	x	x		x	-	-	x	x	x	x	x			10	5	1
EURGen-RLC-011 LR	-	x	x	-	-	-	x			-	-	x	x	-	x	x			6	9	1
EURGen-RLC-014	x	x	x	-	-	-	x	x	x	x	x	x	x	x	-	x	x		11	4	2
EURGen-RLC-014 LR	x	x	x	-	-	-	x	x	x	x	x	x	x	x	-	x	x		11	4	2
EURGen-RLC-015	-	x	-	x	x	x	x	-	-	-	-	x	-	-	-				6	9	
EURGen-RLC-016	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x			15	0	1
EURGen-RLC-017	x	x	x	x	x	x	x	x	x	x	x	x	x	x	-	x			14	1	1
EURGen-RLC-018	-	x	x	x	x	x	x	-	x	-	-	x	x	x	x	x			11	4	1
EURGen-RLC-018 LR	-	x	x	x	x	x	x	-	x	-	-	x	x	x	x	x			11	4	1
EURGen-RLC-019	-	x	x	x	x	x	x	-	x	-	-	x	x	x	x	x			11	4	1
EURGen-RLC-020	-	x	x	x	x	x	x	-	x	-	-	x	x	x	x				11	4	
EURGen-RLC-021	-	x	x	x	x	x	x	-		-	-	x	x	-	x				9	6	
EURGen-RLC-022	-	x	x	x	x	x	x	-	x	-	-	x	x	x	x	x			11	4	1
EURGen-RLC-023	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x			15	0	1
EURGen-RLC-024 LR	-	x	x	x	x	x	x	-	x	-	-	x	x	x	x	x			11	4	1
EURGen-RLC-026	-	x	-	x	x	x	x	-	-	-	-	x	x	-	x	x			8	7	1
EURGen-RLC-027	x	x	x	x	x	x	x	x	x	-	-	x	x	x	x	x			13	2	1
EURGen-RLC-028	-	x	-	x	x	x	x	-	x	-	-	x	x	x	x	x			10	5	1
EURGen-RLC-029	-	x	x	x	x	x	x	-	x	-	-	x	x	x	x	x			11	4	1
EURGen-RLC-030	-	x	x	x	x	x	x	-	x	-	-	x	x	x	x	x			11	4	1
EURGen-RLC-031	x	x	x	x	x	x	x	x	-	x	x	x	x	x	x			x	14	1	1
EURGen-RLC-009																			NA	NA	NA
EURGen-RLC-012																			NA	NA	NA
EURGen-RLC-032																			NA	NA	NA
Correct (nr.)	8	31	27	28	28	28	31	8	25	7	7	31	29	26	26	NA	NA	NA			
Missing or unexpected (nr.)	24	1	5	4	4	4	1	24	7	25	25	1	3	6	6	28	2	1			

Very Major Errors of high significance:

- ○ Almost all missed resistance toward carbapenems
- ○ blaOXA-436 has an incomplete phenotype in ResFinder

⇩

Results must be evaluated critically:

- Results can / should be confirmed with other tools
- Very Major Errors can have important clinical implications
- Points to insufficient knowledge of genetic AMR mechanisms

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OTHER GENERAL RECOMMENDATIONS

For discrepancies due to variations between the type of data and the chosen bioinformatics tools and databases:

- Laboratories planning to implement or in the process of implementing WGS-based analysis in their settings should aim at using harmonized protocols such as the one created during the EURGen-RefLabCap project;
- Laboratories currently using WGS could consider aligning their own protocols with other harmonized protocols;
- 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.

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OTHER GENERAL RECOMMENDATIONS

For discrepancies due to misinterpretation of the EQA protocol and/or insufficient knowledge about certain genetic elements:

- Laboratories should ensure at least basic, and ideally extensive, knowledge about the genetic mechanisms mediating AMR and other important genetic elements;
- Laboratories should be familiar with the mode of action of the bioinformatics tools they use, and the contents of the respective databases;
- Laboratories should analyse their data with the understanding that, currently, there is no "fit-for-all" approach and some types of data and some suites of bioinformatics tools are more adequate for certain purposes than others;
- Laboratories should analyse their results critically and, when needed, perform confirmatory testing, to ensure that the information being reported is accurate and actionable.

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OTHER GENERAL RECOMMENDATIONS

For everyone:

- Continue to participate in genomic EQAs
- Use well-defined QC parameters and respective thresholds
- Use benchmarking datasets to validate the bioinformatics approaches

- Repeat the analysis of the EQA materials and update the bioinformatics pipelines to ensure detection of all genetic determinants

- Contact the EQA organizers for troubleshooting

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Upcoming task

Email consultation for the report

Document will be distributed in late June including updated individual evaluation reports

2 weeks of email consultation period

Changes will be implemented and the updated document will be shared by email

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Thank you on behalf of the EURGen-RefLabCap team

EURGen-RefLabCap@food.dtu.dk