EURGen-RefLabCap

Report from the first external quality assessment exercise

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Third EU Health Programme

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1. INTRODUCTION

1.1. Background

The EURGen-RefLabCap project is complementary to the European Centre of Disease Prevention and Control (ECDC) European Antimicrobial Resistance Genes Surveillance Network (EURGen-Net). The project aims at improving capacities of National Reference Laboratories (NRLs) in European countries for identification and for phenotypic and genotypic characterization of carbapenem-resistant *Enterobacterales* (CRE) and colistin-resistant CREs (CCRE), as well as carbapenem- and/or colistin-resistant *Pseudomonas aeruginosa* (C/CRPa) and *Acinetobacter baumannii* complex (C/CRAb). Furthermore, the project aims at strengthening capacities for national surveillance and outbreak investigation of CRE/CCRE, C/CRPa and C/CRAb, and improve the availability and quality of European-level molecular surveillance data. One of the main goals of the EURGen-RefLabCap project is to support modernisation of diagnostic and molecular typing tests using whole-genome sequencing (WGS) analytical methods in order to achieve those respective aims.

External quality assessment (EQA) exercises are an important tool to assess the capacity of laboratories to follow their own routine procedures and obtain accurate results. This assessment is done by comparing the achieved results with expected results produced by standard methods, and with results obtained by other laboratories. EQAs may also allow for comparing the performance and accuracy of different laboratory protocols and pipelines for analysis of WGS data. This can be possible if the results submitted by participants, for the same type of analyses, are obtained through the use of different methods.

1.2. EQAs in the EURGen-RefLabCap project

Within the EURGen-RefLabCap project, three EQAs are planned (Figure 1) to evaluate and ensure the quality and comparability of the WGS-based data on resistome profiling and high-risk clone identification produced by the NRLs for CRE/CCRE (workstream 1 (WS1) pathogens), and C/CRPa and C/CRAb (workstream 2 (WS2) pathogens). The main objective of the EURGen-RefLabCap EQAs is to assess the laboratories' proficiency regarding WGS and bioinformatics analysis of the relevant pathogens. Results obtained by the participants are compared with the expected results obtained by the Technical University of Denmark (DTU) and Statens Serum Institut (SSI) to assess if WGS-based analysis results are reliable and of consistently good quality. Results from the EQAs will help in planning relevant guidance and training, and potentially encourage laboratories in addressing shortcomings related to their individual results.

WGS data have not yet been properly validated to be used for clinical diagnostic purposes. Some of the analyses included in the EURGen-RefLabCap EQAs have important limitations when considering their applicability in clinical microbiology laboratories, such as the *in silico* prediction of AMR profiles. Thus, the EURGen-RefLabCap EQAs are not an assessment of laboratories' capacity or ability to accurately perform their routine confirmatory, diagnostics or surveillance procedures. Instead, the EQAs aim at comparing bioinformatics approaches used by the NRLs in Europe, to benchmark the performance of those approaches, to identify potential problems or variation between the applied pipelines, and to identify local, national and European opportunities for quality improvement and harmonization of analysis of WGS data.

The EURGen-RefLabCap EQAs are planned in an order of increasing challenge (Figure 1). The first EQA includes WS1 pathogens and encompasses analysis of WGS data through the use of the routine bioinformatics approaches applied by the participating laboratories, with the aim of assessing the accuracy and completeness of those approaches. The second

EQA, focusing on WS1 and WS2 pathogens, also includes DNA sequencing, to furthermore evaluate the capacity for WGS in the individual laboratories and to analyse the quality of locally-produced WGS data. The final EQA, again focusing on WS1 and WS2 pathogens, includes the handling of live bacterial isolates and DNA extraction, to also assess local capacity for those steps of the sequencing process.

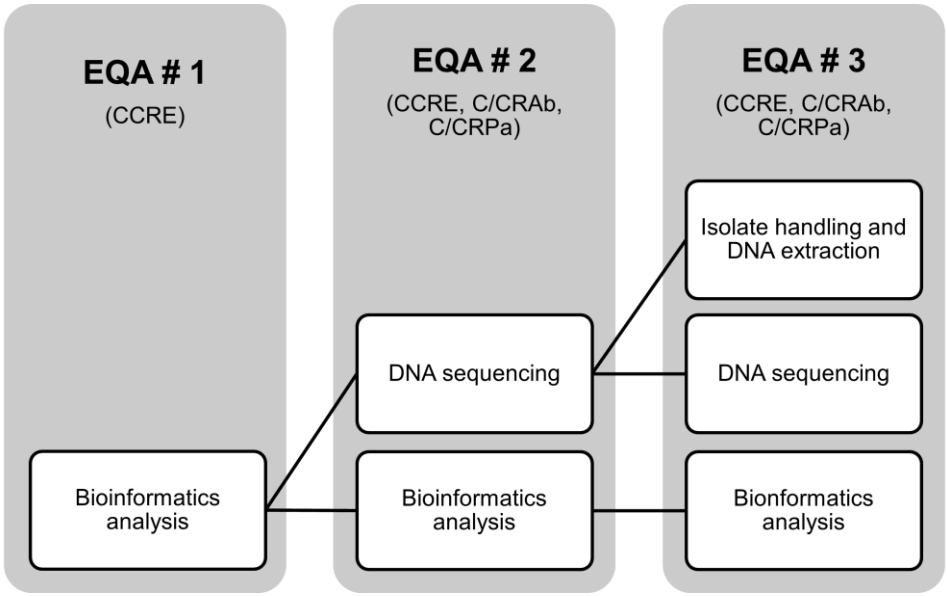

Figure 1. Representation of the three EQAs planned in the EURGen-RefLabCap project

The first EURGen-RefLabCap EQA included raw and assembled sequence data of four strains (two *Escherichia coli* and two *Klebsiella pneumoniae* strains), produced with short-read sequencing technologies (Illumina, Inc., San Diego, CA, United States of America) and long-read sequencing technologies (Oxford Nanopore Technologies, Inc., Oxford, United Kingdom). The EQA included: i) prediction of multi-locus sequence types (MLST); ii) detection of plasmid replicon types; iii) detection of genes and chromosomal point mutations (PMs) mediating antimicrobial resistance (AMR), and; iv) *in silico* prediction of the AMR profiles. All NRLs that participate in the EURGen-RefLabCap project (n=39) were invited to complete the EQA exercise, and 29 laboratories submitted their results.

2. STUDY DESIGN AND METHODS

2.1. EQA material

The material for the EURGen-RefLabCap 2022 EQA corresponded to sequence data obtained from two *E. coli* (EURGen-2022-01 and EURGen-2022-04) and two *K. pneumoniae* (EURGen-2022-02 and EURGen-2022-03) strains. These strains were selected based on their genomic content, including MLST, plasmid replicons and genetic determinants associated with resistance towards carbapenems, colistin and other antimicrobials of clinical relevance. For each strain, participants received two sets of sequence data: one assembled data file (FASTA file) produced with short-read sequencing technologies (Illumina, Inc., San Diego, CA, United States of America), and one assembled data file (FASTA file) produced with long-read sequencing technologies (Oxford Nanopore Technologies, Inc., Oxford, United Kingdom). In case participants were unable to analyse FASTA files, two pairs of raw sequence data files (FASTQ files) for each strain were also available upon request (Table 1).

Table 1. Overview of material available to the participants for the EURGen-RefLabCap 2022 EQA

Strain	Material code	Description
EURGen-2022-01 (*E. coli*)	EURGen-2022-01-FASTA-sr.fasta	Assembled file produced with short-read sequencing
	EURGen-2022-01-FASTA-lr.fasta	Assembled file produced with long-read sequencing
	EURGen_2022_01_FASTQ_sr_R1.fastq.qz EURGen_2022_01_FASTQ_sr_R2.fastq.qz	Raw data files produced with short-read sequencing
	EURGen_2022_01_FASTQ_lr_R1.fastq.qz EURGen_2022_01_FASTQ_lr_R2.fastq.qz	Raw data files produced with long-read sequencing
EURGen-2022-02 (*K. pneumoniae*)	EURGen-2022-02-FASTA-sr.fasta	Assembled file produced with short-read sequencing
	EURGen-2022-02-FASTA-lr.fasta	Assembled file produced with long-read sequencing
	EURGen_2022_02_FASTQ_sr_R1.fastq.qz EURGen_2022_02_FASTQ_sr_R2.fastq.qz	Raw data files produced with short-read sequencing
	EURGen_2022_02_FASTQ_lr_R1.fastq.qz EURGen_2022_02_FASTQ_lr_R2.fastq.qz	Raw data files produced with long-read sequencing
EURGen-2022-03 (*K. pneumoniae*)	EURGen-2022-03-FASTA-sr.fasta	Assembled file produced with short-read sequencing
	EURGen-2022-03-FASTA-lr.fasta	Assembled file produced with long-read sequencing
	EURGen_2022_03_FASTQ_sr_R1.fastq.qz EURGen_2022_03_FASTQ_sr_R2.fastq.qz	Raw data files produced with short-read sequencing
	EURGen_2022_03_FASTQ_lr_R1.fastq.qz EURGen_2022_03_FASTQ_lr_R2.fastq.qz	Raw data files produced with long-read sequencing
EURGen-2022-04 (*E. coli*)	EURGen-2022-04-FASTA-sr.fasta	Assembled file produced with short-read sequencing
	EURGen-2022-04-FASTA-lr.fasta	Assembled file produced with long-read sequencing
	EURGen_2022_04_FASTQ_sr_R1.fastq.qz EURGen_2022_04_FASTQ_sr_R2.fastq.qz	Raw data files produced with short-read sequencing
	EURGen_2022_04_FASTQ_lr_R1.fastq.qz EURGen_2022_04_FASTQ_lr_R2.fastq.qz	Raw data files produced with long-read sequencing

The sequence data were prepared at DTU and at SSI through the use of different DNA extraction kits and protocols, library preparation kits and protocols, sequencing platforms, and quality control and assembly of sequence data strategies. These specifications are described in Table 2.

Table 2. Methods used to generate the material for the EURGen-RefLabCap 2022 EQA

Material code	EURGen-2022-01-sr and EURGen-2022-03-sr and EURGen-2022-04-sr	EURGen-2022-02-sr	EURGen-2022-01-lr and EURGen-2022-04-lr	EURGen-2022-02-lr and EURGen-2022-03-lr
DNA extraction kit	MagnaPure 96 DNA and Viral NA	Qiagen DNeasy Blood & Tissue Kit	GenFind V3	MagnaPure
DNA extraction protocol	MagnaPure DNA Blood ds SV	*In house* modified protocol based on DNeasy® Blood & Tissue Handbook	GenFind V3	MagnaPure
Library preparation kit	Nextera XT DNA Sample Preparation Kit	Nextera XT library Preparation Kit	SQK-RBK004	SQK-RBK110.96
Library preparation protocol	Illumina DNA library preparation kit protocol (adapted for Hamilton Microlab Star)	Illumina DNA library preparation kit protocol	SQK-RBK004	SQK-RBK110.96
Sequencing platform	Illumina NextSeq 550	Illumina MiSeq	Oxford Nanopore MinIon R10.3/R9.4.1r	Oxford Nanopore MinIon R10.3
Quality control of sequence data	Bifrost pipeline (https://github.com/ssi-dk/bifrost) accessing genome size (1X, 10X), average coverage, species ID and unclassified reads	Bifrost pipeline (https://github.com/ssi-dk/bifrost) accessing genome size (1X, 10X), average coverage, species ID and unclassified reads	Average depth ≥50X, quality score >10, trimming to remove adapters, contamination control with Kraken2	Average depth ≥50X, quality score >10, trimming to remove adapters, contamination control with Kraken2
Assembly of sequence data	Skesa v.2.2	Skesa v.2.2	Unicycler (Nanopore only)	Unicycler (Nanopore only)

2.2. Expected results

Expected bioinformatics analysis results were produced at DTU through the use of a suite of bioinformatics tools and databases available at the Center for Genomic Epidemiology (CGE), using the short-read and long-read sequencing FASTA files:

- Species verification was conducted with command line KmerFinder[1] v3.2, database v2022-04-01;
- MLST were predicted with command line MLST[2] v2.0, database v2022-08-01, using the schemes "*Escherichia coli* #1" and "*Klebsiella pneumoniae*";
- Plasmid replicons were detected with command line PlasmidFinder[3] v2.1, database v2021-11-29, with minimum thresholds for parameters identity: 80% and coverage: 60%;
- AMR genes and chromosomal PMs mediating AMR were determined with command line ResFinder[4] v4.1, ResFinder database v2022-07-19 and PointFinder database v2022-06-30, with minimum thresholds for parameters identity: 80% and coverage: 60%.

Expected results were also generated at SSI. In this case, for each file type (FASTA and FASTQ files produced both by short- and long-read sequencing), results regarding species identification, MLST, plasmid replicons, genes and chromosomal mutations mediating AMR, and prediction of AMR profiles were obtained by using two methods in parallel, including:

- A local pipeline that used Ridom SeqSphere+, PlasmidFinder 2.1 and an in-house AMR database based on ResFinder, with thresholds of 95% coverage in length and 90% identity both for plasmid replicons and AMR genes;
- The online services at the CGE: MLST 2.0, PlasmidFinder 2.1 and ResFinder 4.1, all with default parameters (analyses performed on 3 October 2022).

Furthermore, at SSI, FASTA-sr and FASTA-lr files were analyzed using PathogenWatch online, using default parameters (analyses performed on 3 October 2022).

An additional set of expected results was created at a third institution, Centre Hospitalier Universitaire de Caen Normandie, France, through the analysis of FASTA files produced with short- and long-read sequencing. This laboratory mainly used the CGE tools MLST, PlasmidFinder and ResFinder, with additional information recovered from CARD-RGI[5].

[1] https://cge.food.dtu.dk/services/KmerFinder/

[2] https://cge.food.dtu.dk/services/MLST/

[3] https://cge.food.dtu.dk/services/PlasmidFinder/

[4] https://cge.food.dtu.dk/services/ResFinder/

[5] https://card.mcmaster.ca/analyze/rgi

The consensus expected results were produced by critically evaluating the outcome of the methods used by the three institutions and by arbitrarily choosing thresholds of minimum identity 90% and minimum coverage 90% for identification of plasmid replicons, and minimum identity 90% and minimum coverage 60% for identification of AMR determinants. The expected results are summarised in Tables 3, 4 and 5.

Table 3. Expected MLST results for the material included in the 2022 EQA

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.

Table 4. Expected plasmid replicon results for the material included in the 2022 EQA

Material	**Plasmid replicons**
EURGen-2022-01	IncN2; IncFIB(K)
EURGen-2022-02	IncFII; IncL; IncFII(Yp); IncFIB(K)(pCAV1099-114); Col156; IncFIB(pB171); Col(pHAD28); IncHI1B(pNDM-MAR)[a]
EURGen-2022-03	IncFIB(pQil); IncFIB(K); IncFII(K)
EURGen-2022-04	IncHI2A; IncHI2; IncFII; IncFIB(K); Col(pHAD28)

[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.

Table 5. Expected AMR genes and chromosomal PMs mediating AMR and associated *in silico* prediction of the AMR profiles for the material included in the 2022 EQA

Material	AMR genes and chromosomal PMs	Associated prediction of AMR profiles
EURGen-2022-01	*bla*$_{NDM-1}$	Amoxicillin-clavulanic acid, ampicillin, cefepime, cefotaxime, ceftazidime, ceftazidime-avibactam, ertapenem, imipenem, meropenem, piperacillin-tazobactam
EURGen-2022-02	*bla*$_{CTX-M-15}$, *bla*$_{NDM-1}$, *bla*$_{OXA-1}$, *bla*$_{OXA-48}$, *bla*$_{SHV-11}$, *bla*$_{SHV-12}$, *bla*$_{TEM-1}$[a]	Amoxicillin-clavulanic acid, ampicillin, aztreonam, cefepime, cefotaxime, ceftazidime, ceftazidime-avibactam, ertapenem, imipenem, meropenem, piperacillin-tazobactam
	rmtC, aac(6')-Ib-cr, aac(3)-IIa	Amikacin, gentamicin, tobramycin
	aac(6')-Ib-cr, gyrA S83I, *parC* S80I	Ciprofloxacin
	dfrA17	Trimethoprim
	sul1	Sulfamethoxazole
EURGen-2022-03	*bla*$_{KPC-3}$, *bla*$_{CTX-M-15}$, *bla*$_{OXA-1}$, *bla*$_{OXA-9}$, *bla*$_{TEM-1}$[a], *bla*$_{SHV-28}$	Amoxicillin-clavulanic acid, ampicillin, aztreonam, cefepime, cefotaxime, ceftazidime, ertapenem, imipenem, meropenem, piperacillin-tazobactam
	aac(3)-IIa, aac(6')-Ib-cr	Amikacin, gentamicin, tobramycin
	qnrB1, *aac(6')-Ib-cr*, *gyrA* S83I, *parC* S80I	Ciprofloxacin
	dfrA14	Trimethoprim
	sul2	Sulfamethoxazole
	mgrB::IS*1*[b]	Colistin[b]
EURGen-2022-04	*bla*$_{OXA-10}$, *bla*$_{OXA-436}$, *bla*$_{SHV-12}$, *bla*$_{TEM-1}$[a]	Amoxicillin-clavulanic acid, ampicillin, aztreonam, cefepime, cefotaxime, ceftazidime, ertapenem, imipenem, meropenem, piperacillin-tazobactam
	aac(6')-IIc, *gyrA* S83L	Gentamicin, tobramycin
	qnrA1	Ciprofloxacin
	dfrA19	Trimethoprim
	sul1, sul2	Sulfamethoxazole

[a] Any of the variants *bla*$_{TEM-1A}$, *bla*$_{TEM-1B}$, *bla*$_{TEM-1C}$, *bla*$_{TEM-1D}$

[b] Detection of the transposase insertion *mgrB*::IS*1* that leads to inactivation of *mgrB,* and subsequent inclusion of colistin in the AMR profile of this strain, were expected results but not mandatory

2.3. Distribution and procedure

On 1st September 2022, all laboratories that participate in the EURGen-RefLabCap project (n=39) were contacted by email and invited to participate in the 2022 EQA. The email contained a prenotification letter with a brief description of the exercise and indicated that deadline for signing up was 16th September. Confirmations were received from 21 laboratories until the deadline. On 19th September, the remaining 18 laboratories that participate in the project were sent an email with an extension of the sign up deadline until 22nd September. Following this reminder, another 10 laboratories signed up for the 2022 EQA. In total, 31 laboratories signed up to participate in the 2022 EQA. On 30th September, all EQA participants were sent an email confirming their registration and informing them that the exercise would start soon. On 8th October, all EQA participants received an email with instructions on how to download the sequence data from the online, password-protected platform ScienceData[6], and were informed that the protocol for the EQA and the test forms showing the questions that they would encounter on the webtool for submission of results were directly accessible via the EURGen-RefLabCap website[7]. On 20th October, participants received an email informing that the webtool for submission of results[8] was open, and that submission could take place until the deadline of 31st October at 16:00 CET. This email had attached a guideline to create the password for the webtool and a guideline explaining how to access the webtool and submit the results. Until the deadline for submission of results, 20 out of 31 laboratories had completed the EQA. The remaining 11 laboratories were contacted individually to inquire on the status of their analyses and/or submission, and the deadline was extended according to their needs. The EQA was formally completed on 10 November, with 35 sets of results from 29 laboratories, representing 27 countries.

The webtool for submission of results was developed and hosted by DTU for the purpose of this EQA and future related EQAs.

The participants were asked to predict or detect: i) the MLST; ii) the plasmid replicon types; iii) the AMR genes and/or chromosomal PMs mediating AMR, and; iv) the associated *in silico* prediction of AMR profiles. For the latter two types of analyses (iii and iv), the following antimicrobial agents were included in the EQA: amikacin, amoxicillin-clavulanic acid, ampicillin, aztreonam, cefepime, cefotaxime, ceftazidime, ceftazidime-avibactam, ciprofloxacin, colistin, ertapenem, fosfomycin, gentamicin, imipenem, meropenem, piperacillin-tazobactam, tigecycline, tobramycin, trimethoprim, and sulfamethoxazole.

Participants could decide to analyse a selection of the test material, for example only data belonging to *E. coli*, and could decide to submit a subset of results, for example only MLST and plasmid replicons. Participants were encouraged to use the "EURGen-RefLabCap harmonized common WGS-based genome analysis methods and standard protocols for national CCRE surveillance and integrated outbreak investigations"[9], but were welcome to use other WGS analytical set-ups. Thus, they were also asked to report method-related details in relation to the analysis performed, including the bioinformatics tools, databases and parameters used for sequence analyses and generation of results. In total, each participant could submit eight sets of results: two sets of results for each strain, one obtained with the files produced by short-read sequencing (either FASTA or FASTQ files),

[6] https://sciencedata.dk

[7] https://www.eurgen-reflabcap.eu/resources/eqa

[8] https://eurgen-reflabcap-pt.dtu.dk

[9] https://www.eurgen-reflabcap.eu/resources/wgs-tools

and another obtained with the files produced by long-read sequencing (either FASTA or FASTQ files).

On 20th December, all laboratories that submitted results received an email informing that their individual results were available for download from the webtool, including an attachment with a guide for self-evaluation and interpretation of results. This email also contained a link to a feedback survey about the 2022 EQA, with a deadline of 31st January 2023. Until the deadline, one laboratory completed the feedback survey and supplemented the feedback with comments sent directly by email to the EURGen-ReflabCap EQA providers due to text limitation in the online feedback form. An additional number of the laboratories in priority countries more informally commented on the EQA during the regular meetings with the EURGen-RefLabCap team. In June 2023, participants received a new email for completing an abbreviated feedback survey. Seventeen participants replied to the abbreviated survey.

2.4. Scoring system

2.4.1. Overview of the scoring system

In the webtool, the results submitted by the participants were compared to the expected results. The webtool assigned a score "1" in cases of concordance between reported and expected results, and it assigned a score "0" in cases of discordance between reported and expected results (specifically if participants reported plasmid replicons, AMR genes or chromosomal PMs that were not part of the expected results). Moreover, the webtool assigned a "blank" if the participants missed any genetic determinants that were part of the expected results. A complete description of the scoring system is provided in Table 6.

Table 6. Scoring system applied to the analyses included in the 2022 EQA

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

The maximum possible score that each laboratory could achieve, for each type of analysed data (short-read and/or long-read sequencing data), depended on the number of analyses that they performed and for how many strains they performed those analyses. For each type of analysed data, laboratories that performed all analyses for all strains could obtain as a maximum 63 (or 64) points. Table 7 shows the scores regarding each strain and type of analysis included in the 2022 EQA.

Table 7. Maximum possible score for the laboratories participating in the EQA, per strain and per type of analysis

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

2.4.2. Details on the scoring of in silico *prediction of AMR profiles*

In the scoring system for the 2022 EQA the *in silico* prediction of AMR profiles was evaluated as a single answer. This means that the antimicrobials included in the AMR profiles were not evaluated individually by the webtool, but instead all antimicrobials were evaluated together as one complete AMR profile. To obtain a score of "1" the participants had to correctly identify all antimicrobials that were part of the complete AMR profiles. If participants missed one antimicrobial, or if they included additional antimicrobials that were not part of the expected results, the webtool automatically scored the answer as wrong and attributed a value of "0".

For example, for material EURGen-2022-01, participants were expected to predict AMR towards amoxicillin-clavulanic acid, ampicillin, cefepime, cefotaxime, ceftazidime, ceftazidime-avibactam, ertapenem, imipenem, meropenem and piperacillin-tazobactam (Table 5). If participants reported all expected antimicrobials except meropenem, the webtool assigned a score "0". If participants reported all expected antimicrobials, and additionally colistin, the webtool assigned a score "0". This scoring system was meant to reflect the overall capability to predict complete AMR profiles for bacterial isolates from WGS-based results. However, since the webtool scoring does not allow participants to assess their proficiency regarding genomic analysis of antimicrobial-specific resistance profiles, the scoring is planned to be revised for future EQA exercises.

2.4.3. Manual adjustment of the scoring system

According to the overall scoring system, all unexpected genetic determinants reported by the participants were assigned a score "0". However, during validation of the submitted results, it was noted that this score did not adequately reflect participants' proficiency for bioinformatics analysis of WGS data. This was observed for situations where the reported unexpected genetic determinants were a result of differences between bioinformatics databases, or corresponded to gene variants with very high genetic similarity to the expected genes, or were detectable in FASTQ data but not in FASTA data. The specific situations where a score "0" was deemed inappropriate were:

- Reporting the genes *bla*$_{\text{SHV-64}}$ or *bla*$_{\text{SHV-67}}$ instead of the expected genes *bla*$_{\text{SHV-11}}$ or *bla*$_{\text{SHV-12}}$, in strain EURGen-2022-02 (due to high similarity between variants);

- Reporting the genes *bla*$_{\text{SHV-1}}$ or *bla*$_{\text{SHV-106}}$ instead of the expected gene *bla*$_{\text{SHV-28}}$, in strain EURGen-2022-03 (due to high similarity between variants);

- Reporting the gene *aac(3)-IIe* instead of the expected gene *aac(3)-IIa*, in strains EURGen-2022-02 and EURGen-2022-03 (due to differences between bioinformatics databases);

- Reporting the gene *aac(6')-Ib* instead of the expected gene *aac(6')-IIc*, in strain EURGen-2022-04 (due to differences between bioinformatics databases);

- Reporting a *bla*$_{\text{TEM}}$ variant different from *bla*$_{\text{TEM-1}}$, in strain EURGen-2022-03 (due to high similarity between variants);

- Reporting the plasmid replicon IncHI1B(pNDM-MAR), in strain EURGen-2022-02, when using short-read data (due to differences between FASTQ and FASTA data).

The scoring was manually adjusted in these situations to better reflect participants' ability to achieve the expected results defined for this EQA. The individual evaluation reports for each laboratory were updated, and in these situations, participants no longer received a score "0" (which indicates an error), but instead were assigned a result of "blank" (indicating a discrepancy when compared with the expected results).

Due to the complexity of evaluating WGS-based results, and associated limitations of scoring systems, it is advised that participants complement their individual evaluation reports with this present report.

3. RESULTS

3.1. Participating laboratories and analysed materials

Twenty-nine (n=29) laboratories participated in the EURGen-RefLabCap 2022 EQA and were assigned unique anonymized identification codes. Of these, 23 laboratories (79.3%) used only one type of input files, specifically files produced through short-read technologies (n=22) and long-read technologies (n=1). The remaining six laboratories (20.7%) submitted results obtained with the two types of input files (Table 8). For clarity and simplicity, the results of laboratories that used two types of data are evaluated in this report as independent participations (i.e., as one submission based on the use of short-read sequencing data, and another submission based on the use of long-read sequencing data), yielding a final number of 35 participants.

In total, from the 35 sequence analyses that were performed, 28 (80%) used short-read data and the remaining seven (20%) used long-read data. These data were either assembled sequence files (FASTA files) (n=24) or raw sequence data files (FASTQ files) (n=11) (Table 8).

Table 8. Laboratories that participated in the 2022 EQA and type of data analysed by each

Anonymized laboratory identification code	FASTA-sr	FASTA-lr	FASTQ-sr	FASTQ-lr
EURGen-RLC-001	1	0	0	0
EURGen-RLC-002	0	0	1	0
EURGen-RLC-003	1	0	0	0
EURGen-RLC-004	1	1	0	0
EURGen-RLC-005	0	0	1	0
EURGen-RLC-007	1	1	0	0
EURGen-RLC-008	1	1	0	0
EURGen-RLC-009	0	0	1	0
EURGen-RLC-010	1	0	0	0
EURGen-RLC-011	0	0	1	1
EURGen-RLC-012	1	0	0	0
EURGen-RLC-014	1	1	0	0
EURGen-RLC-015	1	0	0	0
EURGen-RLC-016	1	0	0	0
EURGen-RLC-017	1	0	0	0
EURGen-RLC-018	1	1	0	0
EURGen-RLC-019	0	0	1	0
EURGen-RLC-020	0	0	1	0
EURGen-RLC-021	1	0	0	0
EURGen-RLC-022	0	0	1	0
EURGen-RLC-023	0	0	1	0
EURGen-RLC-024	0	1	0	0
EURGen-RLC-026	1	0	0	0
EURGen-RLC-027	1	0	0	0
EURGen-RLC-028	1	0	0	0
EURGen-RLC-029	1	0	0	0
EURGen-RLC-030	1	0	0	0
EURGen-RLC-031	0	0	1	0
EURGen-RLC-032	0	0	1	0
Number of sequence analyses per category	**18**	**6**	**10**	**1**
Total number of sequence analyses	**35**			

All participants correctly identified the species of the four strains included in the 2022 EQA. Most participants (n=32) submitted results for all four types of analysis included in this EQA, except for three participants that did not submit results for *in silico* prediction of AMR profiles, for any strain (Table 9). For all analyses evaluated in this EQA, the concordance between submitted and expected results varied between 28.8% and 93.2% (Figure 2, Table 9). These percentages of concordance were calculated in respect to the maximum possible score for each set of submitted results (which was the sum of total possible points for the number and type of analyses performed by that participant) (Table 9). The descriptions of analysis-specific results are provided in the following sections.

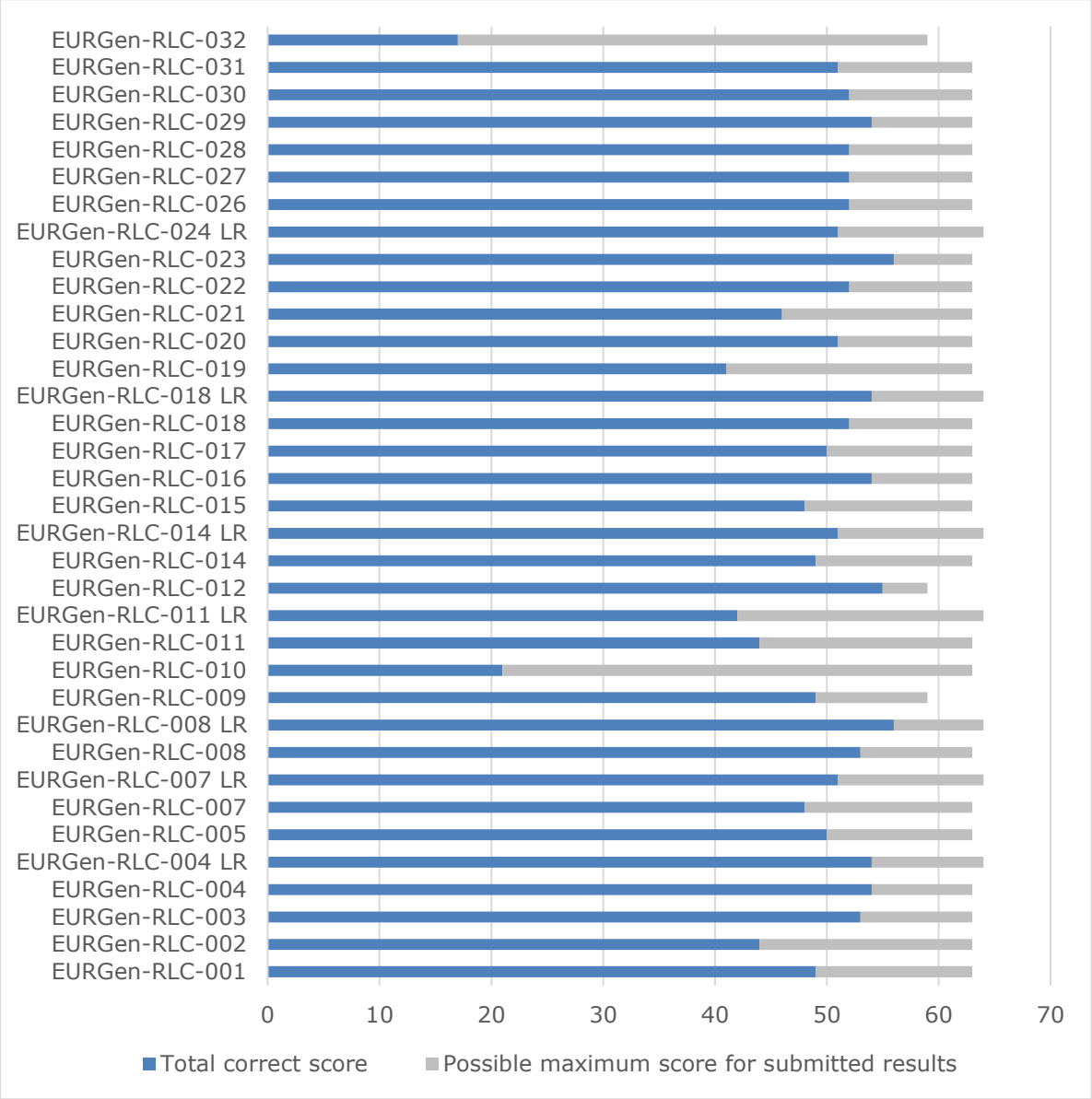

Figure 2. Concordance of submitted and expected results. LR: data produced with long-read sequencing

Table 9. Maximum possible scores and scores obtained by each participant, for each type of analysis included in the 2022 EQA, and in total

Analysis	**Prediction of MLST**			**Detection of plasmid replicons**			**Detection of genetic AMR determinants**			**Prediction of AMR profiles**			**Totals**		
Participants	Maximum possible score	Score	Score (%)	Maximum possible score	Score	Score (%)	Maximum possible score	Score	Score (%)	Maximum possible score	Score	Score (%)	Maximum possible score	Score	Score (%)
EURGen-RLC-001	4	4	100.0	17	8	47.1	38	35	92.1	4	2	50.0	63	49	77.8
EURGen-RLC-002	4	4	100.0	17	14	82.4	38	25	65.8	4	1	25.0	63	44	69.8
EURGen-RLC-003	4	4	100.0	17	13	76.5	38	36	94.7	4	0	0.0	63	53	84.1
EURGen-RLC-004	4	4	100.0	17	16	94.1	38	34	89.5	4	0	0.0	63	54	85.7
EURGen-RLC-004 LR	4	3	75.0	18	17	94.4	38	34	89.5	4	0	0.0	64	54	84.4
EURGen-RLC-005	4	4	100.0	17	13	76.5	38	33	86.8	4	0	0.0	63	50	79.4
EURGen-RLC-007	4	4	100.0	17	13	76.5	38	30	78.9	4	1	25.0	63	48	76.2
EURGen-RLC-007 LR	4	4	100.0	18	14	77.8	38	32	84.2	4	1	25.0	64	51	79.7
EURGen-RLC-008	4	4	100.0	17	13	76.5	38	36	94.7	4	0	0.0	63	53	84.1
EURGen-RLC-008 LR	4	3	75.0	18	15	83.3	38	38	100.0	4	0	0.0	64	56	87.5
EURGen-RLC-009	4	4	100.0	17	14	82.4	38	31	81.6	0	NA	NA	59	49	83.1
EURGen-RLC-010	4	4	100.0	17	13	76.5	38	4	10.5	4	0	0.0	63	21	33.3
EURGen-RLC-011	4	4	100.0	17	13	76.5	38	26	68.4	4	1	25.0	63	44	69.8
EURGen-RLC-011 LR	4	4	100.0	18	15	83.3	38	22	57.9	4	1	25.0	64	42	65.6
EURGen-RLC-012	4	4	100.0	17	17	100.0	38	34	89.5	0	NA	NA	59	55	93.2
EURGen-RLC-014	4	4	100.0	17	13	76.5	38	31	81.6	4	1	25.0	63	49	77.8
EURGen-RLC-014 LR	4	3	75.0	18	15	83.3	38	32	84.2	4	1	25.0	64	51	79.7
EURGen-RLC-015	4	4	100.0	17	13	76.5	38	31	81.6	4	0	0.0	63	48	76.2
EURGen-RLC-016	4	4	100.0	17	13	76.5	38	34	89.5	4	3	75.0	63	54	85.7
EURGen-RLC-017	4	4	100.0	17	12	70.6	38	34	89.5	4	0	0.0	63	50	79.4
EURGen-RLC-018	4	4	100.0	17	13	76.5	38	35	92.1	4	0	0.0	63	52	82.5
EURGen-RLC-018 LR	4	3	75.0	18	15	83.3	38	36	94.7	4	0	0.0	64	54	84.4

Analysis	**Prediction of MLST**			**Detection of plasmid replicons**			**Detection of genetic AMR determinants**			**Prediction of AMR profiles**			**Totals**		
Participants	Maximum possible score	Score	Score (%)	Maximum possible score	Score	Score (%)	Maximum possible score	Score	Score (%)	Maximum possible score	Score	Score (%)	Maximum possible score	Score	Score (%)
EURGen-RLC-019	4	4	100.0	17	3	17.6	38	34	89.5	4	0	0.0	63	41	65.1
EURGen-RLC-020	4	4	100.0	17	14	82.4	38	33	86.8	4	0	0.0	63	51	81.0
EURGen-RLC-021	4	4	100.0	17	11	64.7	38	31	81.6	4	0	0.0	63	46	73.0
EURGen-RLC-022	4	4	100.0	17	13	76.5	38	35	92.1	4	0	0.0	63	52	82.5
EURGen-RLC-023	4	4	100.0	17	17	100.0	38	32	84.2	4	3	75.0	63	56	88.9
EURGen-RLC-024 LR	4	3	75.0	18	11	61.1	38	37	97.4	4	0	0.0	64	51	79.7
EURGen-RLC-026	4	4	100.0	17	13	76.5	38	34	89.5	4	1	25.0	63	52	82.5
EURGen-RLC-027	4	4	100.0	17	13	76.5	38	34	89.5	4	1	25.0	63	52	82.5
EURGen-RLC-028	4	4	100.0	17	13	76.5	38	35	92.1	4	0	0.0	63	52	82.5
EURGen-RLC-029	4	4	100.0	17	13	76.5	38	36	94.7	4	1	25.0	63	54	85.7
EURGen-RLC-030	4	4	100.0	17	13	76.5	38	35	92.1	4	0	0.0	63	52	82.5
EURGen-RLC-031	4	4	100.0	17	10	58.8	38	36	94.7	4	1	25.0	63	51	81.0
EURGen-RLC-032	4	4	100.0	17	0	0.0	38	13	34.2	0	NA	NA	59	17	28.8
Averages	NA	**3.9**	**96.4**	NA	**12.7**	**74.0**	NA	**31.7**	**83.3**	NA	**0.6**	**14.8**	NA	**48.8**	**77.6**

LR: data produced with long-read sequencing; NA: Not applicable

3.2. Prediction of multi-locus sequence types

Participants used both publicly available and commercial software and/or databases for prediction of the MLST. The most commonly reported software was CGE MLST and its respective database. A full description of the methods reported by the participants is provided in Appendix 1.

In total, 140 results were submitted regarding the prediction of MLST, by all participating laboratories. The submitted results were distributed equally between the four strains (n=35 results per strain). Moreover, 80% of the results were obtained using short-read technologies (n=112, or n=28 results per strain), and the remaining 20% of results (n=28, or n=7 results per strain) were obtained using long-read technologies (Table 10, Figure 3).

Of the submitted 140 MLST predictions, 96.4% were correct (n=135). These included all results submitted for strains EURGen-2022-01 (n=35), EURGen-2022-03 (n=35) and EURGen-2022-04 (n=35), regardless of type of file used for analysis, and most (n=30) of the results submitted for strain EURGen-2022-02 (Table 10, Figure 3).

The five incorrect results (3.6%) were submitted for strain EURGen-2022-02, and all were obtained with FASTA files produced through long-read technologies. These discordances were due to the absence of a perfect hit for the *gapA* loci for this strain, which was also detected in the expected results when using FASTA-lr data (Table 3). These results were left empty (or reported as an MLST "0") by the participants, as proposed in the EQA protocol.

Overall, 30 participants correctly identified the MLST of all strains. Participants obtained between three and four points for the identification of MLST, which corresponded to 75.0% to 100.0% of their maximum possible scores (four points for each participant). The average concordance between expected and submitted results was 96.4% (Table 9, Figure 3).

Table 10. Distribution of submitted results regarding the prediction of MLST

Material	**Short-read sequence data**		**Long-read sequence data**		**Total**
Results	**Correct MLST**	**Empty MLST**	**Correct MLST**	**Empty MLST**	
EURGen-2022-01	28	0	7	0	35
EURGen-2022-02	28	0	2	5	35
EURGen-2022-03	28	0	7	0	35
EURGen-2022-04	28	0	7	0	35
Total	112	0	23	5	140

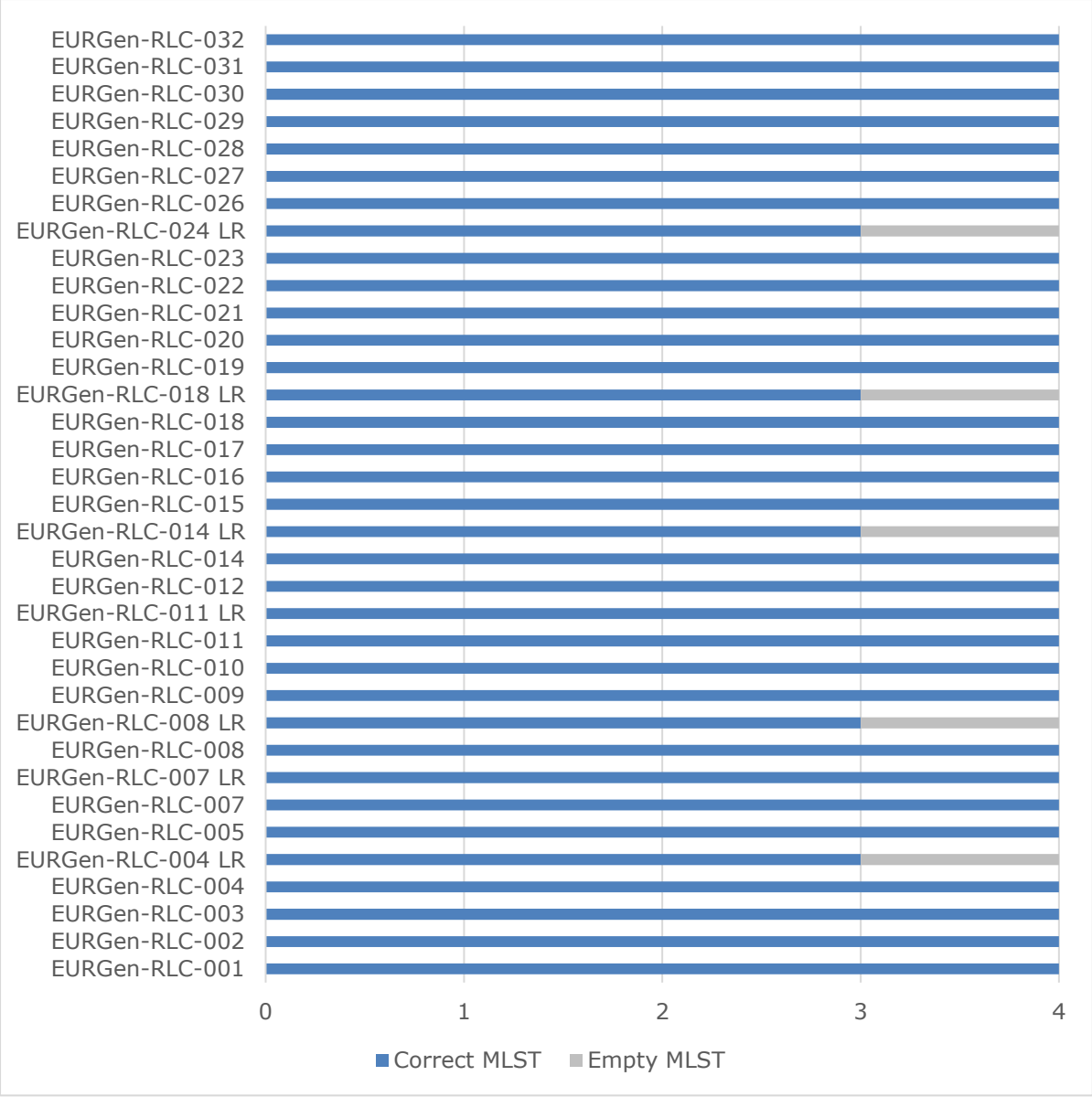

Figure 3. Distribution of submitted results regarding the prediction of MLST. LR: data produced with long-read sequencing

3.3. Detection of plasmid replicon types

Participants used both publicly available and commercial software and/or databases for detection of the plasmid replicons. The most commonly reported software was CGE PlasmidFinder and its respective database. A full description of the methods reported by the participants is provided in Appendix 2.

In total, 140 sets of results were submitted regarding the detection of plasmid replicon types, by all participating laboratories. The submitted results were distributed equally between the four strains (n=35 sets of results per strain). Moreover, 80% of the sets of results were obtained using short-read technologies (n=112, or n=28 results per strain), and the remaining 20% of the sets of results (n=28, or n=7 results per strain) were obtained using long-read technologies (Table 11, Figure 4).

Of the 140 sets of results submitted for detection of plasmid replicons, 48.6% were fully correct (n=68). Additionally, in 47.9% of the sets of results (n=67), certain expected plasmid replicons were missing, and in 18.6% of the submitted results (n=26), unexpected replicons that were not part of the expected results were reported. In some of these cases, the sets of results were missing certain expected replicons and simultaneously contained unexpected replicons (15.0% or n=21) (Table 11, Figure 4).

Overall, two participants correctly identified all expected replicons, and one participant failed to identify any of the expected replicons. Participants obtained between zero and 17 points for the detection of plasmid replicons, which corresponded to 0.0% to 100.0% of their maximum possible scores (17 points for each participant, with one extra point for those analysing long-read sequencing data due to the presence of one extra expected plasmid replicon in those data, when compared with short-read data). The average concordance between expected and submitted results was 74.0% (Table 9, Figure 4).

Table 11. Distribution of submitted results regarding the detection of plasmid replicons

Material	**Short-read sequence data**			**Long-read sequence data**			**Total**
Results	**Correct replicons**	**Missing replicons**	**Unexpected replicons**	**Correct replicons**	**Missing replicons**	**Unexpected replicons**	
EURGen-2022-01	24	3	2[a]	7	0	0	35
EURGen-2022-02	2	25[b]	12[b]	1	6[c]	2[c]	35
EURGen-2022-03	20	7[d]	3[d]	6	1	0	35
EURGen-2022-04	4	23[e]	5[e]	4	2[f]	2[f]	35
Total	50	58	22	18	9	4	140

[a] One set of results contained simultaneously missing and unexpected replicons
[b] Eleven sets of results contained simultaneously missing and unexpected replicons
[c] Two sets of results contained simultaneously missing and unexpected replicons
[d] Two sets of results contained simultaneously missing and unexpected replicons
[e] Four sets of results contained simultaneously missing and unexpected replicons
[f] One set of results contained simultaneously missing and unexpected replicons

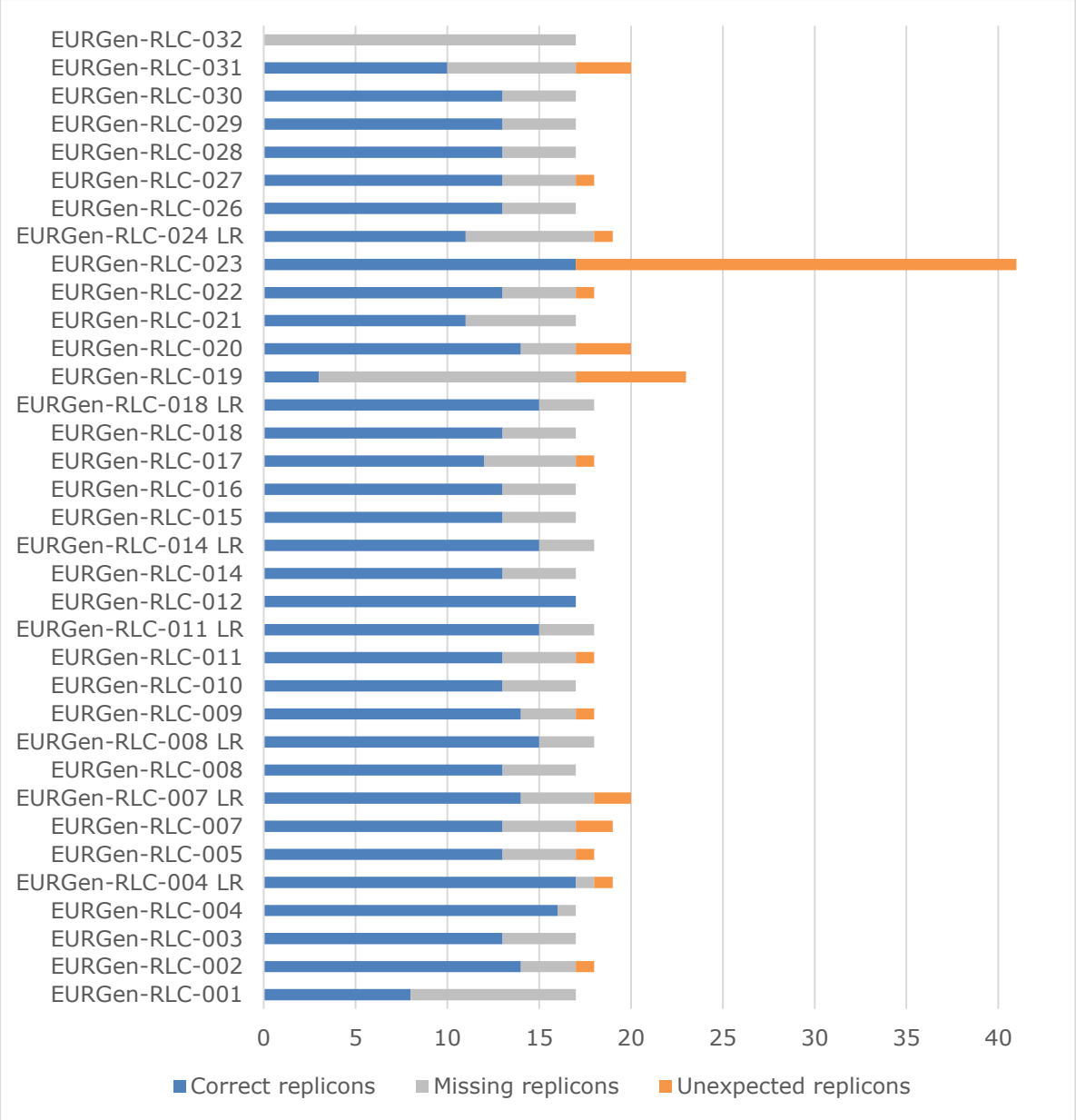

Figure 4. Distribution of submitted results regarding the detection of plasmid replicons. LR: data produced with long-read sequencing

For strain EURGen-2022-01, participants were expected to detect two plasmid replicons (IncN2 and IncFIB(K)). In one set of submitted results the replicon IncFIB(K) was not reported, and in two sets of submitted results neither replicon was reported. The total number of missing replicons throughout all sets of submitted results was five. Overall, 32 sets of results contained all expected plasmid replicons. Furthermore, in one set of submitted results three additional unexpected replicons were reported (repB, IncFIB(K)(pCAV1099-114) and IncN3), and in one set of submitted results the unexpected replicon IncN was reported. All these discordances were reported in cases where short-read sequence data were analysed, and no discordances were reported for analysis performed with long-read sequence data. The total number of unexpected replicons throughout all sets of submitted results was four. A complete description of the concordances and discordances between the expected plasmid replicons and the results submitted by participants is provided in Table 12.

Table 12. Results of the detection of plasmid replicons for each participant, for strain EURGen-2022-01 (*E. coli*)

Laboratories	IncN2	IncFIB(K)	IncN (UN)	IncN3 (UN)	IncFIB(K)(pCAV1099-114) (UN)	repB (UN)	Correct (nr.)	Missing (nr.)	UN (nr.)
EURGen-RLC-001	x	-					1	1	
EURGen-RLC-002	x	x					2	0	
EURGen-RLC-003	x	x					2	0	
EURGen-RLC-004	x	x					2	0	
EURGen-RLC-004 LR	x	x					2	0	
EURGen-RLC-005	x	x					2	0	
EURGen-RLC-007	x	x					2	0	
EURGen-RLC-007 LR	x	x					2	0	
EURGen-RLC-008	x	x					2	0	
EURGen-RLC-008 LR	x	x					2	0	
EURGen-RLC-009	x	x					2	0	
EURGen-RLC-010	x	x					2	0	
EURGen-RLC-011	x	x					2	0	
EURGen-RLC-011 LR	x	x					2	0	
EURGen-RLC-012	x	x					2	0	
EURGen-RLC-014	x	x					2	0	
EURGen-RLC-014 LR	x	x					2	0	
EURGen-RLC-015	x	x					2	0	
EURGen-RLC-016	x	x					2	0	
EURGen-RLC-017	x	x					2	0	
EURGen-RLC-018	x	x					2	0	
EURGen-RLC-018 LR	x	x					2	0	
EURGen-RLC-019	-	-	x				0	2	1
EURGen-RLC-020	x	x					2	0	
EURGen-RLC-021	x	x					2	0	
EURGen-RLC-022	x	x					2	0	
EURGen-RLC-023	x	x		x	x	x	2	0	3
EURGen-RLC-024 LR	x	x					2	0	
EURGen-RLC-026	x	x					2	0	
EURGen-RLC-027	x	x					2	0	
EURGen-RLC-028	x	x					2	0	
EURGen-RLC-029	x	x					2	0	
EURGen-RLC-030	x	x					2	0	
EURGen-RLC-031	x	x					2	0	
EURGen-RLC-032	-	-					0	2	
Correct (nr.)	33	32	NA	NA	NA	NA	**Totals**		
Missing or UN (nr.)	2	3	1	1	1	1	65	5	4

Cells shaded in green (x): Plasmid replicon reported
Cells shaded in red (-): Plasmid replicon missing
Cells shaded in orange (x): Unexpected plasmid replicon reported
LR: data produced with long-read sequencing; NA: Not applicable; UN: Unexpected

For strain EURGen-2022-02, participants were expected to detect seven plasmid replicons (IncFII, IncL, IncFII(Yp), IncFIB(K)(pCAV1099-114), Col156, IncFIB(pB171) and Col(pHAD28)). Additionally, if using data produced by long-read sequencing technologies, participants were also expected to detect the replicon IncHI1B(pNDM-MAR). In 31 sets of submitted results, the expected replicon Col(pHAD28) was not reported, including six sets of results obtained with long-read sequencing data. In 27 sets of submitted results, the expected replicon Col156 was not reported, including five sets of results obtained with long-read sequencing data, and the same distribution of results was observed for replicon IncFIB(pB171). In nine sets of submitted results, the expected replicon IncFIB(K)(pCAV1099-114) was not reported, including two sets of results obtained with long-read sequencing data. There were 11 more cases of other missing replicons from submitted results. The total number of missing replicons throughout all sets of submitted results was 105. Overall, four sets of results contained all expected plasmid replicons. 23 sets of results were missing between one and three replicons. Four other sets of results were missing four replicons, and the remaining four sets of results were missing five, six or all expected plasmid replicons (only one set of results was missing all expected replicons). Unexpected plasmid replicons were reported by 14 laboratories, including analyses performed with short-read (n=12) and long-read (n=2) data. The most commonly reported unexpected replicon was IncHI1B(pNDM-MAR) (n=8) in short-read data, although it was only expected in long-read data. However, this replicon could also be detected in short-read data when using FASTQ files, which was the case for all the participants that unexpectedly reported it. The other commonly reported unexpected replicon was IncFIB(K) (n=5), also in short-read data. The total number of unexpected replicons throughout all sets of submitted results was 26 (Table 13).

Table 13. Results of the detection of plasmid replicons for each participant, for strain EURGen-2022-02 (*K. pneumoniae*)

Laboratories	IncFII	IncL	IncFII(Yp)	IncFIB(K)(pCAV1099-114)	Col156	IncFIB(pB171)	Col(pHAD28)	IncHI1B(pNDM-MAR)	IncFIB(K) (UN)	ColRNAI (UN)	IncFIA (UN)	IncHI1B(R27) (UN)	Other (UN)	Correct (nr.)	Missing (nr.)	UN (nr.)
EURGen-RLC-001	x	x	-	x	-	-	-							3	4	
EURGen-RLC-002	x	x	x	x	-	-	-	x						4	3	1
EURGen-RLC-003	x	x	x	x	-	-	-							4	3	
EURGen-RLC-004	x	x	x	x	x	x	x							7	0	
EURGen-RLC-004 LR	x	x	x	x	x	x	x	x						8	0	
EURGen-RLC-005	x	x	x	x	-	-	-	x						4	3	1
EURGen-RLC-007	x	-	x	-	x	x	-		x					4	3	1
EURGen-RLC-007 LR	x	-	x	-	x	x	-	x	x					5	3	1
EURGen-RLC-008	x	x	x	x	-	-	-							4	3	
EURGen-RLC-008 LR	x	x	x	x	-	-	-	x						5	3	
EURGen-RLC-009	x	x	x	x	x	x	-	x						6	1	1
EURGen-RLC-010	x	x	x	x	-	-	-							4	3	
EURGen-RLC-011	x	x	x	x	-	-	-	x						4	3	1
EURGen-RLC-011 LR	x	x	x	x	-	-	-	x						5	3	
EURGen-RLC-012	x	x	x	x	x	x	x							7	0	
EURGen-RLC-014	x	x	x	x	-	-	-							4	3	
EURGen-RLC-014 LR	x	x	x	x	-	-	-	x						5	3	
EURGen-RLC-015	x	x	x	x	-	-	-							4	3	
EURGen-RLC-016	x	x	x	x	-	-	-							4	3	
EURGen-RLC-017	x	x	x	-	-	-	-		x					3	4	1
EURGen-RLC-018	x	x	x	x	-	-	-							4	3	
EURGen-RLC-018 LR	x	x	x	x	-	-	-	x						5	3	
EURGen-RLC-019	x	x	-	-	-	-	-	x		x	x			2	5	3
EURGen-RLC-020	x	x	x	-	x	x	-	x	x					5	2	2
EURGen-RLC-021	x	x	-	x	-	-	-							3	4	
EURGen-RLC-022	x	x	x	x	-	-	-	x						4	3	1
EURGen-RLC-023	x	x	x	x	x	x	x	x	x				x (a)	7	0	10
EURGen-RLC-024 LR	-	-	x	-	-	-	-	x	x					2	6	1
EURGen-RLC-026	x	x	x	x	-	-	-							4	3	
EURGen-RLC-027	x	x	x	-	-	-	-		x					3	4	1
EURGen-RLC-028	x	x	x	x	-	-	-							4	3	
EURGen-RLC-029	x	x	x	x	-	-	-							4	3	
EURGen-RLC-030	x	x	x	x	-	-	-							4	3	
EURGen-RLC-031	x	x	-	-	-	-	-					x		2	5	1
EURGen-RLC-032	-	-	-	-	-	-	-							0	7	
Correct (nr.)	33	31	30	26	8	8	4	7 /7	NA	NA	NA	NA	NA	**Totals**		
Missing or UN (nr.)	2	4	5	9	27	27	31	8 /28	7	1	1	1	8	147	105	26

Cells shaded in green (x): Plasmid replicon reported
Cells shaded in red (-): Plasmid replicon missing
Cells shaded in orange (x): Unexpected plasmid replicon reported
LR: data produced with long-read sequencing; NA: Not applicable; UN: Unexpected
(a) IncFIB(S), IncFIC(FII), IncFII(29), IncFII(p14), IncFII(pCoo), IncFII(pSE11), IncM2, repB

For strain EURGen-2022-03, participants were expected to detect three plasmid replicons (IncFIB(pQil), IncFIB(K) and IncFII(K)). In two sets of submitted results, none of the expected replicons were detected. In two other sets of results, the replicon IncFIB(K) was missing. In four additional sets of results, the replicon IncFII(K) was missing, including one set of results obtained with long-read sequencing data. The laboratory that could not detect this replicon in long-read data was also unable to detect it in short-read data. The total number of missing replicons throughout all sets of submitted results was 12. Overall, 27 sets of results contained all expected replicons. Six sets of results were missing one plasmid replicon. The remaining two sets of results were missing all expected replicons. Furthermore, in three sets of submitted results, additional unexpected replicons were reported (repB, IncFIB(K)(pCAV1099-114) and IncFII). The total number of unexpected replicons throughout all sets of submitted results was four (Table 14).

Table 14. Results of the detection of plasmid replicons for each participant, for strain EURGen-2022-03 (*K. pneumoniae*)

Laboratories	IncFIB(pQil)	IncFIB(K)	IncFII(K)	IncFII (UN)	IncFIB(K)(pCAV1099-114)	repB (UN)	Correct (nr.)	Missing (nr.)	UN (nr.)
EURGen-RLC-001	x	-	x				2	1	
EURGen-RLC-002	x	x	x				3	0	
EURGen-RLC-003	x	x	x				3	0	
EURGen-RLC-004	x	x	-				2	1	
EURGen-RLC-004 LR	x	x	-				2	1	
EURGen-RLC-005	x	x	x				3	0	
EURGen-RLC-007	x	x	x				3	0	
EURGen-RLC-007 LR	x	x	x				3	0	
EURGen-RLC-008	x	x	x				3	0	
EURGen-RLC-008 LR	x	x	x				3	0	
EURGen-RLC-009	x	x	-				2	1	
EURGen-RLC-010	x	x	x				3	0	
EURGen-RLC-011	x	x	x				3	0	
EURGen-RLC-011 LR	x	x	x				3	0	
EURGen-RLC-012	x	x	x				3	0	
EURGen-RLC-014	x	x	x				3	0	
EURGen-RLC-014 LR	x	x	x				3	0	
EURGen-RLC-015	x	x	x				3	0	
EURGen-RLC-016	x	x	x				3	0	
EURGen-RLC-017	x	x	x				3	0	
EURGen-RLC-018	x	x	x				3	0	
EURGen-RLC-018 LR	x	x	x				3	0	
EURGen-RLC-019	-	-	-	x			0	3	1
EURGen-RLC-020	x	x	x				3	0	
EURGen-RLC-021	x	-	x				2	1	
EURGen-RLC-022	x	x	x				3	0	
EURGen-RLC-023	x	x	x		x	x	3	0	2
EURGen-RLC-024 LR	x	x	x				3	0	
EURGen-RLC-026	x	x	x				3	0	
EURGen-RLC-027	x	x	x				3	0	
EURGen-RLC-028	x	x	x				3	0	
EURGen-RLC-029	x	x	x				3	0	
EURGen-RLC-030	x	x	x				3	0	
EURGen-RLC-031	x	x	-	x			2	1	1
EURGen-RLC-032	-	-	-				0	3	
Correct (nr.)	33	31	29	NA	NA	NA	**Totals**		
Missing or UN (nr.)	2	4	6	2	1	1	93	12	4

Cells shaded in green (x): Plasmid replicon reported
Cells shaded in red (-): Plasmid replicon missing
Cells shaded in orange (x): Unexpected plasmid replicon reported
LR: data produced with long-read sequencing; NA: Not applicable; UN: Unexpected

For strain EURGen-2022-04, participants were expected to detect five plasmid replicons (IncHI2A, IncHI2, IncFII, IncFIB(K) and Col(pHAD28)). In 25 sets of submitted results, the expected replicon Col(pHAD28) was not reported, including two sets of results obtained with long-read sequencing data. In three sets of results, three, four or five replicons were missing. The total number of missing replicons throughout all sets of submitted results

was 34. Overall, ten sets of results contained all expected replicons. 22 sets of results were missing one plasmid replicon. The remaining three sets of results were missing three, four or all expected replicons. Furthermore, in six sets of submitted results, additional unexpected replicons were reported, including in two sets obtained with long-read data. The most commonly reported unexpected replicon was Col440I (n=5). The total number of unexpected replicons throughout all sets of submitted results was 15 (Table 15).

Table 15. Results of the detection of plasmid replicons for each participant, for strain EURGen-2022-04 (*E. coli*)

Laboratories	IncHI2A	IncHI2	IncFII	IncFIB(K)	Col(pHAD28)	Col440I (UN)	IncFIA (UN)	IncN (UN)	Other (UN)	Correct (nr.)	Missing (nr.)	UN (nr.)
EURGen-RLC-001	x	x	-	-	-					2	3	
EURGen-RLC-002	x	x	x	x	x					5	0	
EURGen-RLC-003	x	x	x	x	-					4	1	
EURGen-RLC-004	x	x	x	x	x					5	0	
EURGen-RLC-004 LR	x	x	x	x	x	x				5	0	1
EURGen-RLC-005	x	x	x	x	-					4	1	
EURGen-RLC-007	x	x	x	x	-	x				4	1	1
EURGen-RLC-007 LR	x	x	x	x	-	x				4	1	1
EURGen-RLC-008	x	x	x	x	-					4	1	
EURGen-RLC-008 LR	x	x	x	x	x					5	0	
EURGen-RLC-009	x	x	x	x	-					4	1	
EURGen-RLC-010	x	x	x	x	-					4	1	
EURGen-RLC-011	x	x	x	x	-					4	1	
EURGen-RLC-011 LR	x	x	x	x	x					5	0	
EURGen-RLC-012	x	x	x	x	x					5	0	
EURGen-RLC-014	x	x	x	x	-					4	1	
EURGen-RLC-014 LR	x	x	x	x	x					5	0	
EURGen-RLC-015	x	x	x	x	-					4	1	
EURGen-RLC-016	x	x	x	x	-					4	1	
EURGen-RLC-017	x	x	x	x	-					4	1	
EURGen-RLC-018	x	x	x	x	-					4	1	
EURGen-RLC-018 LR	x	x	x	x	x					5	0	
EURGen-RLC-019	x	-	-	-	-		x			1	4	1
EURGen-RLC-020	x	x	x	x	-	x				4	1	1
EURGen-RLC-021	x	x	x	x	-					4	1	
EURGen-RLC-022	x	x	x	x	-					4	1	
EURGen-RLC-023	x	x	x	x	x	x		x	x (a)	5	0	9
EURGen-RLC-024 LR	x	x	x	x	-					4	1	
EURGen-RLC-026	x	x	x	x	-					4	1	
EURGen-RLC-027	x	x	x	x	x					5	0	
EURGen-RLC-028	x	x	x	x	-					4	1	
EURGen-RLC-029	x	x	x	x	-					4	1	
EURGen-RLC-030	x	x	x	x	-					4	1	
EURGen-RLC-031	x	x	x	x	-			x		4	1	1
EURGen-RLC-032	-	-	-	-	-					0	5	
Correct (nr.)	34	33	32	32	10	NA	NA	NA	NA	**Totals**		
Missing or UN (nr.)	1	2	3	3	25	5	1	2	7	141	34	15

Cells shaded in green (x): Plasmid replicon reported
Cells shaded in red (-): Plasmid replicon missing
Cells shaded in orange (x): Unexpected plasmid replicon reported
LR: data produced with long-read sequencing; NA: Not applicable; UN: Unexpected
(a) IncFIB(K)(pCAV1099-114), IncFIC(FII), IncFII(29), IncFII(pCoo), IncFII(pSE11), pENTAS02, repB

3.4. Detection of genes and chromosomal point mutations mediating AMR

Participants used both publicly available and commercial software and/or databases for detection of the genetic determinants mediating AMR. The most commonly reported software was CGE ResFinder and its respective database. A full description of the methods reported by the participants is provided in Appendix 3.

In total, 140 sets of results were submitted regarding the detection of genetic determinants mediating AMR, by all participating laboratories. The submitted results were distributed equally between the four strains (n=35 sets of results per strain). Moreover, 80% of the sets of results were obtained using short-read technologies (n=112, or n=28 results per strain), and the remaining 20% of the sets of results (n=28, or n=7 results per strain) were obtained using long-read technologies (Table 16, Figure 5).

Of the 140 sets of results submitted for detection of genetic determinants mediating AMR, 24.3% were fully correct (n=34). Additionally, in 58.6% of the sets of results (n=82), certain expected genetic determinants were missing, and in 72.1% of the submitted results (n=101), unexpected genetic determinants that were not part of the expected results were reported. In some of these cases, the sets of results were missing certain expected determinants and simultaneously contained unexpected genetic determinants of AMR (55.0% or n=77) (Table 16, Figure 5).

Overall, only one participant correctly detected all expected genetic determinants of AMR, and there were no participants that failed to identify any of the expected determinants. Participants obtained between four and 38 points for the detection of genetic determinants of AMR, which corresponded to 10.5% to 100.0% of their maximum possible scores (38 points for each participant). The average concordance between expected and submitted results was 83.3% (Table 9, Figure 5).

Table 16. Distribution of submitted results regarding the detection of genetic determinants of AMR

Material	**Short-read sequence data**			**Long-read sequence data**			**Total**
Results	**Correct determinants**	**Missing determinants**	**Unexpected determinants**	**Correct determinants**	**Missing determinants**	**Unexpected determinants**	
EURGen-2022-01	26	0	2	7	0	0	35
EURGen-2022-02	0	28[a]	27[a]	0	4[b]	7[b]	35
EURGen-2022-03	0	25[c]	26[c]	0	5[d]	7[d]	35
EURGen-2022-04	1	15[e]	26[e]	0	5[f]	6[f]	35
Total	27	68	81	7	14	20	140

[a] Twenty-seven sets of results contained simultaneously missing and unexpected determinants
[b] Four sets of results contained simultaneously missing and unexpected determinants
[c] Twenty-three sets of results contained simultaneously missing and unexpected determinants
[d] Five sets of results contained simultaneously missing and unexpected determinants
[e] Fourteen sets of results contained simultaneously missing and unexpected determinants
[f] Four sets of results contained simultaneously missing and unexpected determinants

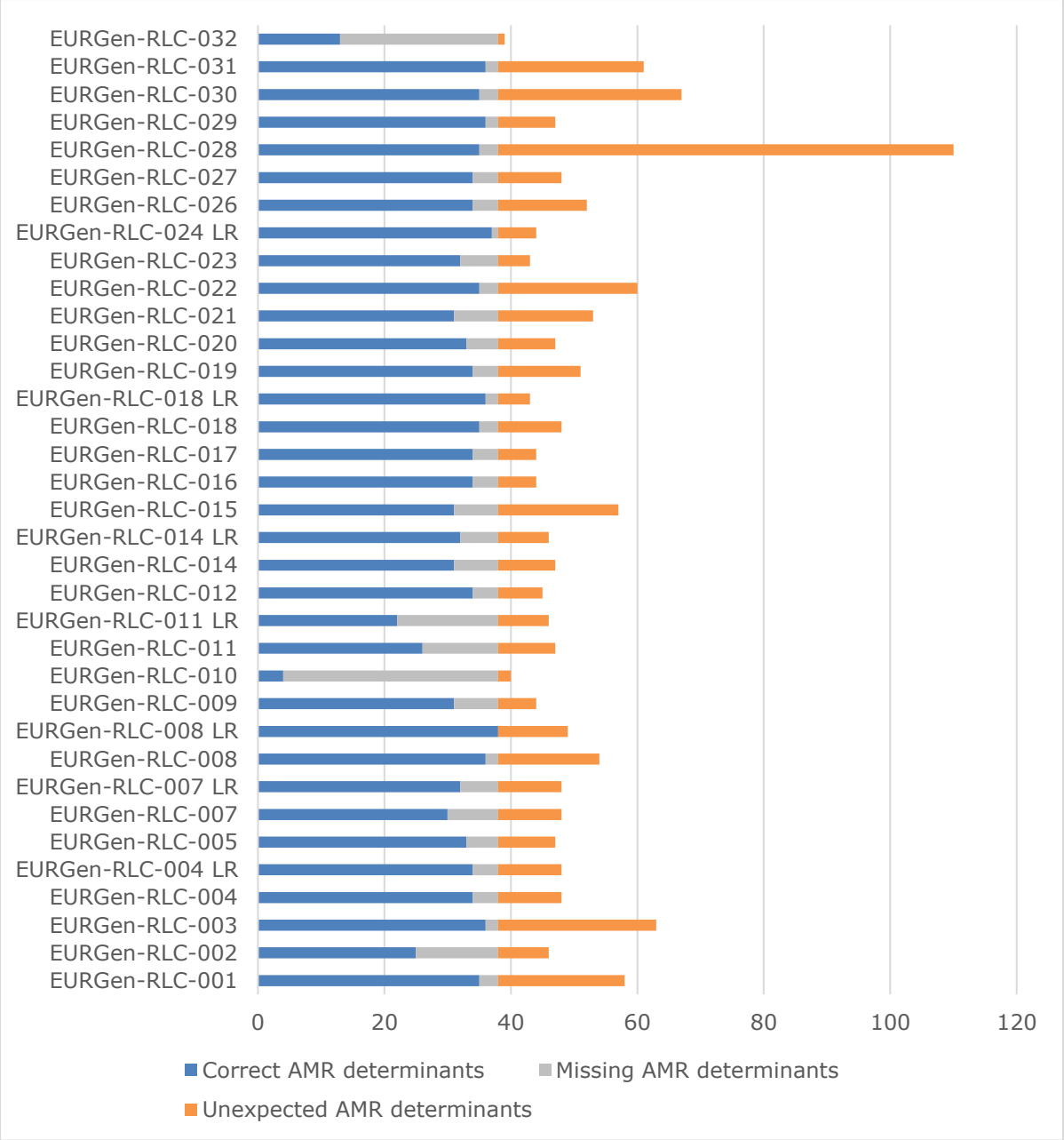

Figure 5. Distribution of submitted results regarding the detection of genetic determinants mediating AMR. LR: data produced with long-read sequencing

For strain EURGen-2022-01, participants were expected to detect one gene mediating AMR (*bla*$_{\text{NDM-1}}$). All participating laboratories were able to detect the expected gene, regardless of the type of data used for analysis. Two laboratories reported unexpected genes or chromosomal PMs, when using short-read data. The total number of unexpected genetic determinants of AMR throughout all sets of submitted results was seven. The remaining 33 sets of results were fully correct, and no unexpected genes or chromosomal PMs were reported. A complete description of the concordances and discordances between the expected genetic determinants of AMR and the results submitted by participants is provided in Table 17.

Table 17. Results of the detection of genetic AMR determinants for each participant, for strain EURGen-2022-01 (*E. coli*)

Laboratories	blaNDM-1	mdt(A) (UN)	Other PMs (UN)	Correct (nr.)	Missing (nr.)	UN (nr.)
EURGen-RLC-001	x			1	0	
EURGen-RLC-002	x			1	0	
EURGen-RLC-003	x			1	0	
EURGen-RLC-004	x			1	0	
EURGen-RLC-004 LR	x			1	0	
EURGen-RLC-005	x			1	0	
EURGen-RLC-007	x			1	0	
EURGen-RLC-007 LR	x			1	0	
EURGen-RLC-008	x			1	0	
EURGen-RLC-008 LR	x			1	0	
EURGen-RLC-009	x	x		1	0	1
EURGen-RLC-010	x			1	0	
EURGen-RLC-011	x			1	0	
EURGen-RLC-011 LR	x			1	0	
EURGen-RLC-012	x			1	0	
EURGen-RLC-014	x			1	0	
EURGen-RLC-014 LR	x			1	0	
EURGen-RLC-015	x			1	0	
EURGen-RLC-016	x			1	0	
EURGen-RLC-017	x			1	0	
EURGen-RLC-018	x			1	0	
EURGen-RLC-018 LR	x			1	0	
EURGen-RLC-019	x			1	0	
EURGen-RLC-020	x			1	0	
EURGen-RLC-021	x			1	0	
EURGen-RLC-022	x			1	0	
EURGen-RLC-023	x			1	0	
EURGen-RLC-024 LR	x			1	0	
EURGen-RLC-026	x			1	0	
EURGen-RLC-027	x			1	0	
EURGen-RLC-028	x		x (a)	1	0	6
EURGen-RLC-029	x			1	0	
EURGen-RLC-030	x			1	0	
EURGen-RLC-031	x			1	0	
EURGen-RLC-032	x			1	0	
Correct (nr.)	35	NA	NA	**Totals**		
Missing or UN (nr.)	0	1	6	35	0	7

Cells shaded in green (x): Genetic AMR determinant reported
Cells shaded in red (-): Genetic AMR determinant missing
Cells shaded in orange (x): Unexpected genetic AMR determinant reported
LR: data produced with long-read sequencing; NA: Not applicable; UN: Unexpected
(a) *parC* E62K (n=1), *pmrB* L279M (n=1), *pmrA* A42D (n=1), 16S-*rrsB* A80C (n=1), 16S-*rrsB* T89G (n=1), 16S-*rrsB* T93C (n=1)

For strain EURGen-2022-02, participants were expected to detect 12 genes mediating AMR (*bla*$_{CTX-M-15}$, *bla*$_{NDM-1}$, *bla*$_{OXA-1}$, *bla*$_{OXA-48}$, *bla*$_{SHV-11}$, *bla*$_{SHV-12}$, *bla*$_{TEM-1}$, *rmtC*, *aac(6')-Ib-cr*, *aac(3)-IIa*, *dfrA17* and *sul1*) and two chromosomal PMs (*gyrA* S83I and *parC* S80I). In 25 sets of submitted results, the expected gene *bla*$_{SHV-11}$ was not reported, including one set of results obtained with long-read sequencing data. In 15 sets of submitted results, the expected gene *aac(3)-IIa* was not reported, including three sets of results obtained with long-read sequencing data. In seven sets of submitted results, all obtained with short-read sequence data, the expected gene *sul1* was missing. The expected chromosomal PM *parC* S80I was missing from 12 sets of results, and the expected PM *gyrA* S83I was missing from six sets of results, and both situations were observed for both types of analysed data. There were 20 more cases of other missing genetic determinants from submitted results. The total number of missing genetic determinants of AMR throughout all sets of submitted results was 85. Overall, only one set of results contained all expected genetic determinants. 31 sets of results were missing between one and four genes or chromosomal PMs. The remaining three sets of results were missing five, eight or 12 expected genetic determinants of AMR. No set of results was missing all the expected determinants of AMR. Unexpected genetic determinants of AMR were reported by almost all laboratories (n=34). The most commonly reported unexpected AMR gene was *fosA* (n=22), followed by *oqxA* (n=20), *oqxB* (n=19) and *aac(3)-IIe* (n=10). Unexpected chromosomal PMs were especially frequent for the genes *acrR* (n=59), *pmrB* (n=11) and *ramR* (n=11), although others were observed. The total number of unexpected genetic determinants of AMR throughout all sets of submitted results was 176 (Table 18).

Table 18. Results of the detection of genetic AMR determinants for each participant, for strain EURGen-2022-02 (*K. pneumoniae*)

Laboratories	blaCTX-M-15	blaNMD-1	blaOXA-1	blaOXA-48	blaSHV-11	blaSHV-12	blaTEM-1	rmtC	aac(6')-Ib-cr	aac(3)-IIa	dfrA17	sul1	gyrA S83I	parC S80I	oqxA (UN)	oqxB (UN)	fosA (UN)	aac(3)-IIe (UN)	aac(6')-Ib (UN)	blaSHV-67 (UN)	blaSHV-64 (UN)	rmtA (UN)	fomA (UN)	PMs in pmrB (UN) (b)	PMs in acrR (UN) (c)	PMs in ramR (UN) (d)	PMs in parC (UN) (e)	PMs in gyrB (UN) (f)	PMs in gyrA (UN) (g)	PMs in 16S-rrsB (UN) (h)	Correct (nr.)	Missing (nr.)	UN (nr.)
EURGen-RLC-001	x	x	x	x	-	x	x	x	x	x	x	x	x	x	x									x	x						13	1	9
EURGen-RLC-002	x	x	x	x	x	x	x	x	x	-	-	-	x	-				x	x					x			x	x			10	4	5
EURGen-RLC-003	x	x	x	x	-	x	x	x	x	x	x	x	x	x			x								x						13	1	8
EURGen-RLC-004	x	x	x	x	-	x	x	x	x	-	x	x	x	x	x	x	x	x						x							12	2	5
EURGen-RLC-004 LR	x	x	x	x	x	x	x	x	x	-	x	x	x	x	x	x	x	x						x							13	1	5
EURGen-RLC-005	x	x	x	x	x	x	x	x	x	x	x	x	x	-	x	x	x				x										13	1	4
EURGen-RLC-007	x	x	x	x	-	x	x	-	x	-	x	x	x	-	x	x	x	x						x							10	4	5
EURGen-RLC-007 LR	x	x	x	x	x	x	x	-	x	-	x	x	x	-	x	x	x	x						x							11	3	5
EURGen-RLC-008	x	x	x	x	-	x	x	x	x	x	x	x	-	x	x	x	x											x			12	2	4
EURGen-RLC-008 LR	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x			x											14	0	4
EURGen-RLC-009	x	x	x	x	-	x	x	-	x	x	x	-	x	x			x					x									11	3	2
EURGen-RLC-010	-	x	-	x	-	-	-	-	-	-	-	-	-	-																x	2	12	1
EURGen-RLC-011	x	x	x	x	x	x	x	x	-	-	x	-	-	-			x								x				x		9	5	3
EURGen-RLC-011 LR	x	x	x	x	-	x	x	x	-	x	x	x	-	-											x		x		x		10	4	3
EURGen-RLC-012	x	x	x	x	-	x	x	x	x	-	x	x	x	-	x	x	x	x													11	3	4
EURGen-RLC-014	x	x	x	x	-	x	x	x	x	-	x	x	x	-	x	x		x						x							11	3	4
EURGen-RLC-014 LR	x	x	x	x	x	x	x	x	x	-	x	x	x	-	x	x		x						x							12	2	4
EURGen-RLC-015	x	x	x	x	-	x	x	x	x	-	x	x	x	x			x								x						12	2	8
EURGen-RLC-016	x	x	x	x	-	x	-	x	x	x	x	x	x	x	x	x															12	2	2
EURGen-RLC-017	x	x	x	x	-	x	x	x	x	-	x	x	x	x			x (a)								x						12	2	2
EURGen-RLC-018	x	x	x	x	-	x	x	x	x	x	x	x	x	x			x														13	1	1
EURGen-RLC-018 LR	x	x	x	x	x	x	x	x	x	x	x	x	x	x			x			x											14	0	2
EURGen-RLC-019	x	x	x	x	x	-	x	x	x	x	-	x	x	x			x			x								x			12	2	3
EURGen-RLC-020	x	x	x	x	-	x	x	x	x	-	x	x	x	x	x	x	x	x													12	2	4
EURGen-RLC-021	x	x	x	x	-	x	x	x	x	x	x	x	x	x											x						13	1	7
EURGen-RLC-022	x	x	x	x	-	-	x	x	x	x	x	x	x	x	x	x	x								x						12	2	10
EURGen-RLC-023	x	x	x	x	-	x	x	x	x	-	x	-	x	x				x						x							11	3	2
EURGen-RLC-024 LR	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x															14	0	2
EURGen-RLC-026	x	x	x	x	-	x	x	x	x	x	x	x	x	x	x	x			x												13	1	3
EURGen-RLC-027	x	x	x	x	-	x	x	x	x	x	x	x	-	x	x	x	x							x					x		12	2	5
EURGen-RLC-028	x	x	x	x	-	x	x	x	x	x	x	-	x	-									x		x	x	x	x			11	3	25
EURGen-RLC-029	x	x	x	x	-	x	x	x	x	x	x	x	x	x	x	x	x												x		13	1	4
EURGen-RLC-030	x	x	x	x	-	x	x	x	x	x	x	x	x	x	x	x	x								x						13	1	10
EURGen-RLC-031	x	x	x	x	-	x	x	x	x	x	x	x	x	x	x	x	x							x	x						13	1	11
EURGen-RLC-032	x	x	x	x	-	x	x	-	-	-	-	-	-	-																	6	8	0
Correct (nr.)	34	35	34	35	10	32	33	30	31	20	31	28	29	23	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA		**Totals**		
Missing or UN (nr.)	1	0	1	0	25	3	2	5	4	15	4	7	6	12	20	19	22	10	2	3	1	1	1	11	59	11	7	4	4	1	405	85	176

Cells shaded in green (x): Genetic AMR determinant reported
Cells shaded in red (-): Genetic AMR determinant missing
Cells shaded in orange (x): Unexpected genetic AMR determinant reported
LR: data produced with long-read sequencing; NA: Not applicable; UN: Unexpected
(a) The laboratory reported the gene *fos* instead of, presumably, *fosA*
(b) *pmrB* R256G (n=11)
(c) *acrR* F172S (n=8), *acrR* F197I (n=8), *acrR* G164A (n=8), *acrR* K201M (n=8), *acrR* L195V (n=8), *acrR* P161R (n=8), *acrR* R173G (n=8), *acrR* (unspecified) (n=3)
(d) *ramR* A16K (n=1), *ramR* A17L (n=1), *ramR* A19P (n=1), *ramR* A20R (n=1), *ramR* A22S (n=1), *ramR* E15E (n=1), *ramR* F21L (n=1), *ramR* G25P (n=1), *ramR* I26A (n=1), *ramR* Q23P (n=1), *ramR* T18P (n=1)
(e) *parC* A171G (n=1), *parC* P170T (n=1), *parC* R173A (n=1), *parC* S129A (n=1), *parC* S172I (n=1), *parC* T169T (n=1), *parC* (unspecified) (n=1)
(f) *gyrB* E466D (n=3), *gyrB* S83I (n=1)
(g) *gyrA* S81I (n=1), *gyrA* S83L (n=1), *gyrA* (unspecified) (n=2)
(h) 16S-*rrsB* (unspecified) (n=1)

For strain EURGen-2022-03, participants were expected to detect 11 genes mediating AMR (*bla*$_{KPC-3}$, *bla*$_{CTX-M-15}$, *bla*$_{OXA-1}$, *bla*$_{OXA-9}$, *bla*$_{TEM-1}$, *bla*$_{SHV-28}$, *aac(3)-IIa*, *aac(6')-Ib-cr*, *qnrB1*, *dfrA14* and *sul2*) and two chromosomal PMs (*gyrA* S83I and *parC* S80I). Additionally, detection of the chromosomal PM *mgrB::*IS*1* was optional (accepted as a correct result but not a requirement for achieving the maximum possible score). In 16 sets of submitted results, the expected gene *aac(3)-IIa* was not reported, including four sets of results obtained with long-read sequencing data. In 15 sets of submitted results, the expected gene *bla*$_{OXA-9}$ was not reported, including three sets of results obtained with long-read sequencing data. In 13 sets of submitted results, the expected gene *bla*$_{SHV-28}$ was not reported, including two sets of results obtained with long-read sequencing data. In eight sets of submitted results, all obtained with short-read sequence data, the expected gene *bla*$_{TEM-1}$ was missing. The expected chromosomal PM *parC* S80I was missing from 11 sets of results, and the expected PM *gyrA* S83I was missing from four sets of results, and both situations were observed for both types of analysed data. There were 19 more cases of other missing genetic determinants from submitted results. The total number of missing genetic determinants of AMR throughout all sets of submitted results was 86. Overall, five sets of results contained all expected genetic determinants. 16 sets of results were missing between one or two genes or chromosomal PMs. Eight sets of results were missing three genetic determinants of AMR, and another three sets of results were missing five determinants. The remaining three sets of results were missing 10 or 12 expected genetic determinants of AMR. No set of results was missing all the expected determinants of AMR. None of the participants reported the optional chromosomal PM *mgrB::*IS*1*. Unexpected genetic determinants of AMR were reported by almost all laboratories (n=32). The most commonly reported unexpected AMR genes were *bla*$_{TEM}$ genes different from *bla*$_{TEM-1}$ (n=47), followed by *fosA* (n=28), *oqxA* (n=21), *oqxB* (n=21) and *aac(3)-IIe* (n=11). Unexpected chromosomal PMs were especially frequent for the genes *acrR* (n=59), *gyrA* (n=14) and *parC* (n=7), although others were observed. The total number of unexpected genetic determinants of AMR throughout all sets of submitted results was 224 (Table 19).

Table 19. Results of the detection of genetic AMR determinants for each participant, for strain EURGen-2022-03 (*K. pneumoniae*)

Laboratories	blaKPC-3	blaCTX-M-15	blaOXA-1	blaOXA-9	blaTEM-1	blaSHV-28	aac(3)-IIa	aac(6')-Ib-cr	qnrB1	dfrA14	sul2	gyrA S83I	parC S80I	mgrB::IS1 (optional)	oqxA (UN)	oqxB (UN)	fosA (UN)	aac(3)-IIe (UN)	aac(6')-Ib (UN)	blaSHV-106 (UN)	blaSHV-1 (UN)	blaSHV-100 (UN)	Other blaTEM (UN) (d)	PMs in acrR (UN) (e)	PMs in gyrA (UN) (f)	PMs in parC (UN) (g)	PMs in ramR (UN) (h)	Correct (nr.)	Missing (nr.)	UN (nr.)
EURGen-RLC-001	x	x	x	-	x	x	x	x	x	x	x	x	x	-	x	x	x							x				12	1	10
EURGen-RLC-002	x	x	x	x	x (a)	x	-	x	-	-	-	x	-	-				x	x							x		8	5	3
EURGen-RLC-003	x	x	x	x	x (a)	x	x	x	x	x	x	x	x	-			x			x			x	x				13	0	16
EURGen-RLC-004	x	x	x	-	x	x	-	x	x	x	x	x	x	-	x	x	x	x										11	2	4
EURGen-RLC-004 LR	x	x	x	-	x	x	-	x	x	x	x	x	x	-	x	x	x	x										11	2	4
EURGen-RLC-005	x	x	x	x	x	-	x	x	x	x	x	x	-	-	x	x	x											11	2	3
EURGen-RLC-007	x	x	x	-	x	x	-	x	x	x	x	x	-	-	x	x	x	x										10	3	4
EURGen-RLC-007 LR	x	x	x	-	x	x	-	x	x	x	x	x	-	-	x	x	x	x										10	3	4
EURGen-RLC-008	x	x	x	x	x (a)	x	x	x	x	x	x	x	x	-	x	x	x			x			x					13	0	11
EURGen-RLC-008 LR	x	x	x	x	x (a)	x	x	x	x	x	x	x	x	-	x	x	x			x								13	0	4
EURGen-RLC-009	x	x	x	x	-	x	x	x	x	x	-	x	x	-			x (b)						x					11	2	2
EURGen-RLC-010	x	-	-	-	-	-	-	-	-	-	-	-	-	-														1	12	0
EURGen-RLC-011	x	x	x	-	x	-	x	-	x	x	x	-	-	-										x	x		x	8	5	3
EURGen-RLC-011 LR	x	x	x	-	x	-	x	-	x	x	x	-	-	-										x	x	x		8	5	3
EURGen-RLC-012	x	x	x	x	x	x	-	x	x	x	x	x	x	-			x	x										12	1	2
EURGen-RLC-014	x	x	x	-	x	x	-	x	x	x	x	x	-	-	x	x	x	x										10	3	4
EURGen-RLC-014 LR	x	x	x	-	x	x	-	x	x	x	x	x	-	-	x	x	x	x										10	3	4
EURGen-RLC-015	x	x	x	x	-	-	-	x	x	x	x	x	x	-			x (b)					x	x	x				10	3	10
EURGen-RLC-016	x	x	x	x	-	x	x	x	x	x	x	x	x	-	x	x	x											12	1	3
EURGen-RLC-017	x	x	x	x	x	-	-	x	x	x	x	x	x	-			x (c)			x				x				11	2	3
EURGen-RLC-018	x	x	x	x	x (a)	-	x	x	x	x	x	x	x	-			x			x			x					12	1	8
EURGen-RLC-018 LR	x	x	x	x	x (a)	-	x	x	x	x	x	x	x	-			x			x								12	1	2
EURGen-RLC-019	x	x	x	x	x	x	x	x	x	-	-	x	x	-	x	x	x			x			x					11	2	9
EURGen-RLC-020	x	x	x	-	x	-	-	x	x	x	x	x	x	-	x	x	x	x			x							10	3	5
EURGen-RLC-021	x	x	x	-	-	-	x	x	x	x	x	x	x	-										x				10	3	7
EURGen-RLC-022	x	x	x	x	x	-	x	x	x	x	x	x	x	-	x	x	x (b)			x				x				12	1	11
EURGen-RLC-023	x	x	x	-	-	x	-	x	x	x	x	x	x	-			x	x										10	3	2
EURGen-RLC-024 LR	x	x	x	x	x (a)	x	x	x	x	x	x	x	x	-	x	x	x											13	0	3
EURGen-RLC-026	x	x	x	x	x (a)	-	x	x	x	x	x	x	x	-	x	x			x	x			x					12	1	10
EURGen-RLC-027	x	x	x	-	x	x	-	x	x	x	x	x	x	-	x	x	x	x										11	2	4
EURGen-RLC-028	x	x	x	x	x (a)	x	x	x	x	x	x	x	x	-	x	x	x			x			x	x	x	x		13	0	34
EURGen-RLC-029	x	x	x	x	x	x	x	x	x	x	x	x	-	-	x	x	x								x			12	1	4
EURGen-RLC-030	x	x	x	x	x (a)	-	-	x	x	x	x	x	x	-	x	x	x			x			x	x				11	2	17
EURGen-RLC-031	x	x	x	x	-	x	x	x	x	x	x	x	x	-	x	x	x						x	x				12	1	11
EURGen-RLC-032	x	x	-	-	-	x	-	-	-	-	-	-	-	-														3	10	0
Correct (nr.)	35	34	33	20	27	22	19	31	32	31	30	31	24	0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA		**Totals**	
Missing or UN (nr.)	0	1	2	15	8	13	16	4	3	4	5	4	11	35	21	21	28	11	2	11	1	1	47	59	14	7	1	369	86	224

Cells shaded in green (x): Genetic AMR determinant reported
Cells shaded in red (-): Genetic AMR determinant missing
Cells shaded in orange (x): Unexpected genetic AMR determinant reported
LR: data produced with long-read sequencing; NA: Not applicable; UN: Unexpected
(a) These participants (n=10) reported simultaneously two variants of the *bla*$_{\text{TEM-1}}$ gene (*bla*$_{\text{TEM-1A}}$, *bla*$_{\text{TEM-1B}}$, *bla*$_{\text{TEM-1C}}$ and/or *bla*$_{\text{TEM-1D}}$); any of these variants would be an adequate choice, but they should not have been reported simultaneously.
(b) These participants (n=3) reported the gene *fosA6*
(c) The participant reported the gene *fos* instead of, presumably, *fosA*
(d) *bla*$_{\text{TEM-57}}$ (n=8), *bla*$_{\text{TEM-135}}$ (n=7), *bla*$_{\text{TEM-141}}$ (n=7), *bla*$_{\text{TEM-29}}$ (n=6), *bla*$_{\text{TEM-55}}$ (n=6), *bla*$_{\text{TEM-122}}$ (n=6), *bla*$_{\text{TEM-209}}$ (n=4), *bla*$_{\text{TEM-206}}$ (n=1), *bla*$_{\text{TEM-214}}$ (n=1), *bla*$_{\text{TEM-216}}$ (n=1)
(e) *acrR* F172S (n=8), *acrR* F197I (n=8), *acrR* G164A (n=8), *acrR* K201M (n=8), *acrR* L195V (n=8), *acrR* P161R (n=8), *acrR* R173G (n=8), *acrR* (unspecified) (n=3)
(f) *gyrA* A331G (n=1), *gyrA* C326L (n=1), *gyrA* P329S (n=1), *gyrA* R327Q (n=1), *gyrA* S325Q (n=1), *gyrA* S328V (n=1), *gyrA* S330F (n=1), *gyrA* S332I (n=1), *gyrA* S80I (n=1), *gyrA* T324T (n=1), *gyrA* T333N (n=1), *gyrA* W334M (n=1), *gyrA* (unspecified) (n=2)
(g) *parC* A171G (n=1), *parC* P170T (n=1), *parC* R173A (n=1), *parC* S129A (n=1), *parC* S172I (n=1), *parC* T169T (n=1), *parC* (unspecified) (n=1)
(h) *ramR* (unspecified) (n=1)

For strain EURGen-2022-04, participants were expected to detect nine genes mediating AMR (*bla*$_{OXA-10}$, *bla*$_{OXA-436}$, *bla*$_{SHV-12}$, *bla*$_{TEM-1}$, *aac(6')-IIc*, *qnrA1*, *dfrA19*, *sul1* and *sul2*) and one chromosomal PM (*gyrA* S83I). In eight sets of submitted results, the expected gene *sul1* was not reported, and in eight sets of results the expected gene *sul2* was not reported. The gene *sul1* was missing only from results obtained with short-read sequence data, but *sul2* was missing from results obtained with both types of data. The expected genes *aac(6')-IIc*, *dfrA19* and *bla*$_{OXA-436}$, as well as the expected chromosomal PM *gyrA* S83L, were missing from six sets of results each. There were 11 other cases of other missing genetic determinants from submitted results. The total number of missing genetic determinants of AMR throughout all sets of submitted results was 51. Overall, 14 sets of results contained all expected genetic determinants. 16 sets of results were missing one or two genes or chromosomal PMs. The remaining five sets of results were missing three, four, six, seven or all expected genetic determinants of AMR. Unexpected genetic determinants of AMR were reported by 31 laboratories. The most commonly reported unexpected AMR gene was *mcr-9* (n=31), followed by unexpected chromosomal PMs in *gyrA* (n=4). The total number of unexpected genetic determinants of AMR throughout all sets of submitted results was 45 (Table 20).

Table 20. Results of the detection of genetic AMR determinants for each participant, for strain EURGen-2022-04 (*E. coli*)

Laboratories	blaOXA-10	blaOXA-436	blaSHV-12	blaTEM-1	aac(6')-IIc	qnrA1	dfrA19	sul1	sul2	gyrA S83L	mcr-9 (UN)	oqxA (UN)	oqxB (UN)	aac(6')-Ib (UN)	aadD (UN)	PMs in gyrA (UN) (a)	Other PMs (UN) (b)	Correct (nr.)	Missing (nr.)	UN (nr.)
EURGen-RLC-001	x	x	x	x	x	x	x	-	x	x	x							9	1	1
EURGen-RLC-002	x	x	x	x	x	-	-	-	-	x								6	4	0
EURGen-RLC-003	x	x	x	x	x	x	-	x	x	x	x							9	1	1
EURGen-RLC-004	x	x	x	x	x	x	x	x	x	x	x							10	0	1
EURGen-RLC-004 LR	x	x	x	x	x	x	x	x	-	x	x							9	1	1
EURGen-RLC-005	x	-	x	x	x	x	x	x	x	-	x					x		8	2	2
EURGen-RLC-007	x	x	x	x	x	x	x	-	x	x	x							9	1	1
EURGen-RLC-007 LR	x	x	x	x	x	x	x	x	x	x	x							10	0	1
EURGen-RLC-008	x	x	x	x	x	x	x	x	x	x	x							10	0	1
EURGen-RLC-008 LR	x	x	x	x	x	x	x	x	x	x	x	x	x					10	0	3
EURGen-RLC-009	x	x	x	x	x	x	x	-	-	x	x							8	2	1
EURGen-RLC-010	-	-	-	-	-	-	-	-	-	-	x							0	10	1
EURGen-RLC-011	x	x	x	x	-	x	x	x	x	-	x			x		x		8	2	3
EURGen-RLC-011 LR	x	x	-	-	-	-	-	x	-	-	x					x		3	7	2
EURGen-RLC-012	x	x	x	x	x	x	x	x	x	x	x							10	0	1
EURGen-RLC-014	x	x	x	x	x	x	x	-	x	x	x							9	1	1
EURGen-RLC-014 LR	x	x	x	x	x	x	x	x	-	x								9	1	0
EURGen-RLC-015	x	x	x	x	x	x	-	x	x	-						x		8	2	1
EURGen-RLC-016	x	x	x	-	x	x	x	x	x	x	x							9	1	1
EURGen-RLC-017	x	x	x	x	x	x	x	x	x	x	x							10	0	1
EURGen-RLC-018	x	-	x	x	x	x	x	x	x	x	x							9	1	1
EURGen-RLC-018 LR	x	-	x	x	x	x	x	x	x	x	x							9	1	1
EURGen-RLC-019	x	x	x	x	x	x	x	x	x	x	x							10	0	1
EURGen-RLC-020	x	x	x	x	x	x	x	x	x	x								10	0	0
EURGen-RLC-021	x	x	x	x	-	-	x	-	x	x	x							7	3	1
EURGen-RLC-022	x	x	x	x	x	x	x	x	x	x	x							10	0	1
EURGen-RLC-023	x	x	x	x	x	x	x	x	x	x	x							10	0	1
EURGen-RLC-024 LR	x	x	x	x	x	x	x	x	-	x	x							9	1	1
EURGen-RLC-026	x	-	x	x	-	x	x	x	x	x	x							8	2	1
EURGen-RLC-027	x	x	x	x	x	x	x	x	x	x	x							10	0	1
EURGen-RLC-028	x	x	x	x	x	x	x	x	x	x	x						x	10	0	7
EURGen-RLC-029	x	x	x	x	x	x	x	x	x	x	x							10	0	1
EURGen-RLC-030	x	x	x	x	x	x	x	x	x	x	x				x			10	0	2
EURGen-RLC-031	x	x	x	x	x	x	x	x	x	x	x							10	0	1
EURGen-RLC-032	x	-	x	x	-	-	-	-	-	-	x							3	7	1
Correct (nr.)	34	29	33	32	29	30	29	27	27	29	NA	NA	NA	NA	NA	NA	NA	**Totals**		
Missing or UN (nr.)	1	6	2	3	6	5	6	8	8	6	31	1	1	1	1	4	6	299	51	45

Cells shaded in green (x): Genetic AMR determinant reported
Cells shaded in red (-): Genetic AMR determinant missing
Cells shaded in orange (x): Unexpected genetic AMR determinant reported
LR: data produced with long-read sequencing; NA: Not applicable; UN: Unexpected
(a) *gyrA* S83I (n=1), *gyrA* P83L (n=1), *gyrA* (unspecified) (n=2)
(b) *parC* E62K (n=1), *pmrB* L279M (n=1), *pmrA* A42D (n=1), 16S-*rrsB* A80C (n=1), 16S-*rrsB* T89G (n=1), 16S-*rrsB* T93C (n=1)

For all four strains, participants had the option of reporting chromosomal PMs leading to upregulation of *ampC* β-lactamase expression. These PMs were not expected in any strain, and were not reported by any participant.

3.5. *In silico* prediction of antimicrobial resistance profiles

3.5.1. Automatic analysis of in silico *prediction of AMR profiles*

In silico prediction of AMR profiles was generally conducted simultaneously with the detection of the genetic determinants mediating AMR (Appendix 3).

In total, 128 results were submitted regarding the *in silico* prediction of AMR profiles, by 32 participants. Three participants did not submit results (EURGen-RLC-009, EURGen-RLC-012 and EURGen-RLC-032). The submitted results were distributed equally between the four strains (n=32 results per strain). Moreover, 78.1% of the results were obtained using short-read technologies (n=100, or n=25 results per strain), and the remaining 21.9% of the results (n=28, or n=7 results per strain) were obtained using long-read technologies (Table 21).

Of the 128 AMR profiles that were submitted, 14.8% were fully correct (n=19). Additionally, in 63.3% of the sets of results (n=81), certain expected antimicrobials were missing. Finally, in 60.9% of the submitted results (n=78), unexpected antimicrobials that were not part of the expected AMR profiles were reported. In some of these cases, the results were missing certain expected antimicrobials and simultaneously contained unexpected antimicrobials (39.1% or n=50) (Table 21).

Overall, none of the participants correctly predicted all expected AMR profiles, and 18 participants failed to predict any of the expected profiles. Participants obtained between zero and three points for the prediction of AMR profiles, which corresponded to 0.0% to 75.0% of their maximum possible scores (four points for each participant). The average concordance between expected and submitted results was 14.8% (Table 9).

Table 21. Distribution of submitted results regarding the *in silico* prediction of AMR profiles

Material	**Short-read sequence data**			**Long-read sequence data**			**Total**
Results	**Correct profiles**	**Missing antimicrobials**	**Unexpected antimicrobials**	**Correct profiles**	**Missing antimicrobials**	**Unexpected antimicrobials**	
EURGen-2022-01	9	16[a]	1[a]	2	5	0	32
EURGen-2022-02	3	18[b]	17[b]	0	5[c]	5[c]	32
EURGen-2022-03	4	6[d]	18[d]	1	1[e]	6[e]	32
EURGen-2022-04	0	23[f]	24[f]	0	7[g]	7[g]	32
Total	16	63	60	3	18	18	128

[a] One result contained simultaneously missing and unexpected antimicrobials
[b] Thirteen sets of results contained simultaneously missing and unexpected antimicrobials
[c] Three results contained simultaneously missing and unexpected antimicrobials
[d] Three results contained simultaneously missing and unexpected antimicrobials
[e] One result contained simultaneously missing and unexpected antimicrobials
[f] Twenty-two results contained simultaneously missing and unexpected antimicrobials
[g] Seven results contained simultaneously missing and unexpected antimicrobials

3.5.2. Manual scoring of in silico *prediction of AMR profiles*

Although the antimicrobials included in the AMR profiles were not scored individually by the webtool, the manual scoring was performed for the purpose of this report. The manual scoring is not included in the individual evaluation reports for each laboratory, therefore they should be complemented with this report.

Overall, two participants correctly included all the expected antimicrobials (n=58) in the AMR profiles, and there were no participants that failed to identify any of the expected antimicrobials. Participants correctly predicted individual AMR profiles for between nine and 58 antimicrobials, which corresponded to 15.5% to 100.0% of all expected antimicrobials. The average concordance between expected and submitted results at the antimicrobial level was 87.6% (Table 22, Figure 6).

Table 22. Maximum possible number of antimicrobials included in the expected complete AMR profiles, and number of reported antimicrobials, for each participant

Participants	**Maximum possible antimicrobials**	**Correctly included antimicrobials**	**Correctly included antimicrobials (%)**
EURGen-RLC-001	58	54	93.1
EURGen-RLC-002	58	50	86.2
EURGen-RLC-003	58	52	89.7
EURGen-RLC-004	58	52	89.7
EURGen-RLC-004 LR	58	52	89.7
EURGen-RLC-005	58	52	89.7
EURGen-RLC-007	58	54	93.1
EURGen-RLC-007 LR	58	54	93.1
EURGen-RLC-008	58	52	89.7
EURGen-RLC-008 LR	58	52	89.7
EURGen-RLC-009	0	NA	NA
EURGen-RLC-010	58	9	15.5
EURGen-RLC-011	58	50	86.2
EURGen-RLC-011 LR	58	47	81.0
EURGen-RLC-012	0	NA	NA
EURGen-RLC-014	58	54	93.1
EURGen-RLC-014 LR	58	53	91.4
EURGen-RLC-015	58	43	74.1
EURGen-RLC-016	58	58	100.0
EURGen-RLC-017	58	55	94.8
EURGen-RLC-018	58	52	89.7
EURGen-RLC-018 LR	58	52	89.7
EURGen-RLC-019	58	50	86.2
EURGen-RLC-020	58	51	87.9
EURGen-RLC-021	58	49	84.5
EURGen-RLC-022	58	51	87.9
EURGen-RLC-023	58	58	100.0
EURGen-RLC-024 LR	58	52	89.7
EURGen-RLC-026	58	49	84.5
EURGen-RLC-027	58	56	96.6
EURGen-RLC-028	58	51	87.9
EURGen-RLC-029	58	53	91.4
EURGen-RLC-030	58	52	89.7
EURGen-RLC-031	58	57	98.3
EURGen-RLC-032	0	NA	NA
Averages	NA	**50.8**	**87.6**

LR: data produced with long-read sequencing; NA: Not applicable

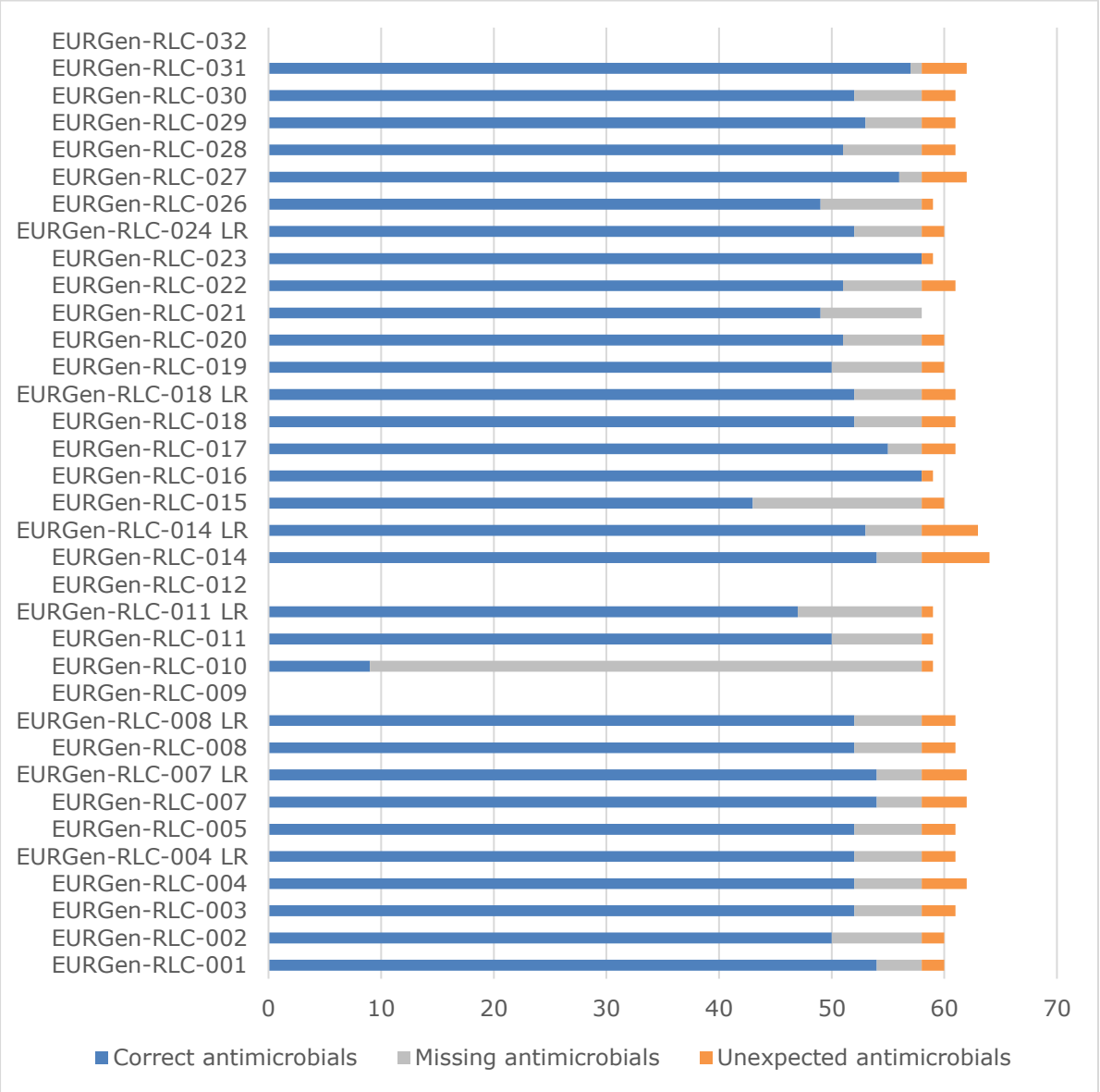

Figure 6. Distribution of submitted results regarding the *in silico* prediction of AMR profiles. LR: data produced with long-read sequencing

For strain EURGen-2022-01, participants were expected to predict resistance towards 10 antimicrobials (amoxicillin-clavulanic acid, ampicillin, cefepime, cefotaxime, ceftazidime, ceftazidime-avibactam, ertapenem, imipenem, meropenem and piperacillin-tazobactam). In 21 submitted results, the expected antimicrobial ceftazidime-avibactam was not reported, including five results obtained with long-read sequencing data. In two submitted results, the expected antimicrobial amoxicillin-clavulanic acid was not reported, and both results were obtained with short-read sequencing data. There were six more cases of other missing antimicrobials, restricted to results submitted by two laboratories that analysed short-read sequencing data. The total number of missing antimicrobials throughout all submitted results was 29. Overall, 11 results contained all expected antimicrobials. 18 results were missing only one antimicrobial, and two results were missing two antimicrobials. The remaining result was missing seven expected antimicrobials. No result

was missing all expected antimicrobials. Only one unexpected antimicrobial was reported, by one participant. A complete description of the concordances and discordances between the expected AMR profiles and the results submitted by participants is provided in Table 23.

Table 23. Results of the *in silico* prediction of AMR profiles for each participant, for strain EURGen-2022-0 1 (*E. coli*)

Laboratories	Amoxicillin-clav	Ampicillin	Cefepime	Cefotaxime	Ceftazidime	Ceftazidime-avib	Ertapenem	Imipenem	Meropenem	Piperacillin-tazob	Fosfomycin (UN)	Correct (nr.)	Missing (nr.)	UN (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	**Totals**		
Missing or UN (nr.)	2	1	1	1	1	21	0	1	0	1	1	291	29	1

Cells shaded in green (x): AMR profile reported for the antimicrobial
Cells shaded in red (-): AMR profile missing for the antimicrobial
Cells shaded in orange (x): AMR profile reported for the unexpected antimicrobial
Cells shaded in grey (/): participant did not submit AMR profile
LR: data produced with long-read sequencing; NA: Not applicable; UN: Unexpected

For strain EURGen-2022-02, participants were expected to predict resistance towards 17 antimicrobials (amoxicillin-clavulanic acid, ampicillin, aztreonam, cefepime, cefotaxime, ceftazidime, ceftazidime-avibactam, ertapenem, imipenem, meropenem, piperacillin-tazobactam, amikacin, gentamicin, tobramycin, ciprofloxacin, trimethoprim and sulfamethoxazole). In 22 submitted results, the expected antimicrobial ceftazidime-avibactam was not reported, including five results obtained with long-read sequencing data. In four submitted results, the expected antimicrobial sulfamethoxazole was not reported, and all those results were obtained with short-read sequencing data. The antimicrobials trimethoprim and ciprofloxacin were missing from three and two, respectively, results obtained with short-read data. There were 10 more cases of other missing antimicrobials, restricted to results submitted by one laboratory that analysed short-read sequencing data. The total number of missing antimicrobials throughout all submitted results was 41. Overall, nine results contained all expected antimicrobials. 18 results were missing only one antimicrobial, and four results were missing two or three antimicrobials. The remaining result was missing 14 expected antimicrobials. No result was missing all expected antimicrobials. Unexpected antimicrobials were reported by several laboratories (n=22). The most commonly reported unexpected antimicrobial was fosfomycin (n=19), followed by colistin (n=7) and tigecycline (n=2). The total number of unexpected antimicrobials throughout all submitted results was 28. The complete description of the results submitted by participants is provided in Table 24.

Table 24. Results of the *in silico* prediction of AMR profiles for each participant, for strain EURGen-2022-02 (*K. pneumoniae*)

Laboratories	Amikacin	Amoxicillin-clav	Ampicillin	Aztreonam	Cefepime	Cefotaxime	Ceftazidime	Ceftazidime-avib	Ciprofloxacin	Ertapenem	Gentamicin	Imipenem	Meropenem	Piperacillin-tazob	Sulfamethoxazole	Tobramycin	Trimethoprim	Fosfomycin (UN)	Colistin (UN)	Tigecycline (UN)	Correct (nr.)	Missing (nr.)	UN (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	**Totals**		
Missing or UN (nr.)	1	1	1	1	1	1	1	22	2	0	1	0	0	1	4	1	3	19	7	2	503	41	28

Cells shaded in green (x): AMR profile reported for the antimicrobial
Cells shaded in red (-): AMR profile missing for the antimicrobial
Cells shaded in orange (x): AMR profile reported for the unexpected antimicrobial
Cells shaded in grey (/): participant did not submit AMR profile
LR: data produced with long-read sequencing; NA: Not applicable; UN: Unexpected

For strain EURGen-2022-03, participants were expected to predict resistance towards 16 antimicrobials (amoxicillin-clavulanic acid, ampicillin, aztreonam, cefepime, cefotaxime, ceftazidime, ertapenem, imipenem, meropenem, piperacillin-tazobactam, amikacin, gentamicin, tobramycin, ciprofloxacin, trimethoprim and sulfamethoxazole). Additionally, prediction of resistance towards colistin was optional (accepted as a correct result but not a requirement for a fully correct AMR profile). In four submitted results, the expected antimicrobial trimethoprim was not reported, including one result obtained with long-read sequencing data. In four submitted results, the expected antimicrobial amikacin was not reported, and all those results were obtained with short-read sequencing data. The antimicrobial sulfamethoxazole was missing from two results obtained with short-read data. There were 10 more cases of other missing antimicrobials, restricted to results submitted by one laboratory that analysed short-read sequencing data. The total number

of missing antimicrobials throughout all submitted results was 20. Overall, 25 results contained all expected antimicrobials. 15 results were missing only one antimicrobial, and one results was missing two antimicrobials. The remaining result was missing 13 expected antimicrobials. No result was missing all expected antimicrobials. None of the participants included the optional antimicrobial colistin in the submitted AMR profile. Unexpected antimicrobials were reported by several laboratories (n=24). The most commonly reported unexpected antimicrobial was fosfomycin (n=23), followed by tigecycline (n=2). The total number of unexpected antimicrobials throughout all submitted results was 25. The complete description of the results submitted by participants is provided in Table 25.

Table 25. Results of the *in silico* prediction of AMR profiles for each participant, for strain EURGen-2022-03 (*K. pneumoniae*)

Laboratories	Amikacin	Amoxicillin-clav	Ampicillin	Aztreonam	Cefepime	Cefotaxime	Ceftazidime	Ciprofloxacin	Ertapenem	Gentamicin	Imipenem	Meropenem	Piperacillin-tazob	Sulfamethoxazole	Tobramycin	Trimethoprim	Colistin (optional)	Fosfomycin (UN)	Tigecycline (UN)	**Correct (nr.)**	**Missing (nr.)**	**UN (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	**Totals**		
Missing or UN (nr.)	4	1	1	1	1	1	1	1	0	1	0	0	1	2	1	4	32	23	2	492	20	25

Cells shaded in green (x): AMR profile reported for the antimicrobial
Cells shaded in red (-): AMR profile missing for the antimicrobial
Cells shaded in orange (x): AMR profile reported for the unexpected antimicrobial
Cells shaded in grey (/): participant did not submit AMR profile
LR: data produced with long-read sequencing; NA: Not applicable; UN: Unexpected

For strain EURGen-2022-04, participants were expected to predict resistance towards 15 antimicrobials (amoxicillin-clavulanic acid, ampicillin, aztreonam, cefepime, cefotaxime, ceftazidime, ertapenem, imipenem, meropenem, piperacillin-tazobactam, gentamicin, tobramycin, ciprofloxacin, trimethoprim and sulfamethoxazole). In 25 submitted results, the expected antimicrobials imipenem and meropenem were not reported, including six results obtained with long-read sequencing data. In all except one of those results (n=24), the antimicrobials ertapenem and amoxicillin-clavulanic acid were also missing. There were 42 more cases of other missing antimicrobials, including gentamicin (n=7), tobramycin (n=6) and trimethoprim (n=6). The total number of missing antimicrobials throughout all submitted results was 140. Overall, only two results contained all expected antimicrobials. Three results were missing only one or two antimicrobials, and 20 results were missing four antimicrobials. Four results were missing between five and seven antimicrobials, and two of the results were missing nine antimicrobials. The remaining result was missing 15 expected antimicrobials. No result was missing all expected antimicrobials. Unexpected antimicrobials were reported by almost all laboratories (n=29). The most commonly reported unexpected antimicrobial was colistin (n=28), followed by tigecycline (n=2) and ceftazidime-avibactam (n=1). The total number of unexpected antimicrobials throughout all submitted results was 31. The complete description of the results submitted by participants is provided in Table 26.

Table 26. Results of the *in silico* prediction of AMR profiles for each participant, for strain EURGen-2022-04 (*E. coli*)

Laboratories	Amoxicillin-clav	Ampicillin	Aztreonam	Cefepime	Cefotaxime	Ceftazidime	Ciprofloxacin	Ertapenem	Gentamicin	Imipenem	Meropenem	Piperacillin-tazob	Sulfamethoxazole	Tobramycin	Trimethoprim	Colistin (UN)	Tigecycline (UN)	Ceftazidime-avib (UN)	Correct (nr.)	Missing (nr.)	UN (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	**Totals**		
Missing or UN (nr.)	24	1	5	4	4	4	1	24	7	25	25	1	3	6	6	28	2	1	340	140	31

Cells shaded in green (x): AMR profile reported for the antimicrobial
Cells shaded in red (-): AMR profile missing for the antimicrobial
Cells shaded in orange (x): AMR profile reported for the unexpected antimicrobial
Cells shaded in grey (/): participant did not submit AMR profile
LR: data produced with long-read sequencing; NA: Not applicable; UN: Unexpected

3.6. Feedback survey

Seventeen participants replied to the abbreviated feedback survey shared in June 2023. Furthermore, oral feedback was received during the EURGen-RefLabCap Network meeting (20-21 June 2023) and during the EURGen-RefLabCap physical workshop (21-22 June 2023).

Participants rated the usefulness of the EQA to their laboratories on a scale from 1 to 10: the average score was 9, with the distribution of scores being 10 (n=9 or 53%), 9 (n=3 or 18%), 8 (n=1 or 6%), 7 (n=1 or 6%), 6 (n=1 or 6%), 5 (n=2 or 12%), and no scores under 5.

Fourteen respondents (82%) answered that the individual EQA evaluation reports they received in December 2022 were clear and useful. Two respondents (12%) answered that the reports were not clear and useful, and one participant (6%) did not reply.

Ten participants (59%) answered that they took corrective action(s) as a result of the individual EQA evaluation reports they received in December 2022, and the remaining seven respondents (41%) answered that they did not take corrective action(s).

Respondents were able to include free text answers regarding any suggestions to make upcoming EQAs more useful. The responses received during the feedback survey were aligned with the oral discussions undertaken during the two physical meetings in June 2023, and can be summarized in six main points:

- There are concerns regarding the accuracy and real-life applicability of the prediction of AMR profiles from genomic data;
- The AMR profiles should be scored for each antimicrobial, instead of as a complete profile;
- The participants would appreciate a longer deadline for submitting results for the EURGen-RefLabCap EQAs;
- There is no overall score in the individual evaluation reports, nor comparison with results from other participants;
- The participants would appreciate receiving certificates of participation;
- The participants would appreciate further activities regarding bioinformatics approaches for analysis of WGS data.

4. DISCUSSION

4.1. Participation in the EQA

Twenty-nine laboratories that participate in the EURGen-RefLabCap project completed the 2022 EQA (74.4% of the 39 invited laboratories). These represented 27 of the 37 countries involved in the project (73.0%). Some laboratories that did not report results for the 2022 EQA might have missed the exercise due to time or personnel constraints, or might be in the process of increasing their capacity for processing of WGS data, therefore might be able to participate in future EQAs.

4.2. Prediction of multi-locus sequence types

The prediction of MLST for all strains, by all participants, was close to full concordance with the expected results. The discrepancies were due to some of the participants that used long-read sequencing files observing the imperfect hit for the *gapA* loci for strain EURGen-2022-02. When this situation occurs during routine laboratory work for diagnostics, confirmatory or surveillance purposes, it is advisable that laboratories report the closest MLST, furthermore being advised to report the imperfect allele to the respective database curators (specifically PubMLST or other institutions responsible for the scheme being used) so that a new MLST can be assigned and be accessible for surveillance or other public health purposes. The discrepancy between results obtained with short- and long-read sequencing data, even with the use of the same or similar bioinformatics tools and databases, also illustrates that there are differences between these technologies and, generally, short-read sequencing data have higher accuracy when used for analyses that are sensitive to single-nucleotide polymorphisms (SNPs).

4.3. Detection of plasmid replicon types

During the detection of plasmid replicons almost half of the submitted sets of results were missing certain expected replicons. The most commonly missed replicon was Col(pHAD28), which was missing from 31 sets of results submitted for strain EURGen-2022-02 and missing from 25 sets of results submitted for strain EURGen-2022-04. This is most likely due to the participants' choice of thresholds, which potentially were stricter than those used to generate the expected results (which were minimum identity of 90% and minimum coverage of 90%). Of note, this choice of thresholds to generate expected results was arbitrary and the use of different thresholds is not necessarily incorrect. Often, the choice of thresholds will vary according to the purpose of the analyses. Other commonly missed replicons were IncFIB(pB171) and Col156, both in strain EURGen-2022-02, which participants failed to report in 27 cases each. The reason is likely the same as explained before.

The plasmid replicon IncHI1B(pNDM-MAR) present in strain EURGen-2022-02 was only part of the expected results for long-read sequencing data, but nevertheless a few participants using short-read sequencing data were able to detect it. These participants used FASTQ files for their analyses, and the replicon could potentially be detected when using those files. However, the replicon was not detectable when using FASTA-sr files, thus it was not included as part of the expected results due to the discrepancy between the short-read datasets. This difference in expected results furthermore consolidates the observation that there are differences between sequencing technologies, with long-read sequencing being overall more adequate for detection of plasmids. Additionally, the assembly process might fail to properly capture sequences that were present in raw data, for example due to a different depth of coverage than the one of the genomic DNA, or due

to sequencing of more fragmented plasmid DNA (as a consequence of the DNA extraction process which often is not optimized for adequate plasmid extraction). Thus, it is important to consider the goals of each analysis before selecting a particular technology or bioinformatics approach.

The reporting of unexpected plasmid replicons happened in 18.6% of submissions, less frequently than missing expected replicons. Many of these situations appeared to be due to selecting a similar but incorrect replicon type, either due to distraction or insufficient knowledge regarding the difference between related replicon types. For example, the unexpected replicon IncFIB(K) was frequently reported instead of the correct replicon IncFIB(K)(pCAV1099-114) in strain EURGen-2022-02.

One laboratory reported 24 unexpected plasmid replicons throughout the results submitted for the four strains. This laboratory used the most commonly used bioinformatics tool (PlasmidFinder) and stated to use strict thresholds for selection of the plasmid replicons, which is not in agreement with the very high number of unexpected replicons reported. This situation supports the approach of confirming results of one bioinformatics tool by the use of additional tools. It furthermore shows the importance of the critical evaluation of bioinformatics results, to ensure that relevant data do not become lost within the "noise" of other hits of poor quality.

4.4. Detection of genes and chromosomal point mutations mediating AMR

During the detection of genetic determinants of AMR, almost 60% of submitted sets of results were missing expected determinants. The most commonly missed genes were those encoding β-lactamases. In specific, genes belonging to the *bla*$_{SHV}$ family (n=43 times, i.e., *bla*$_{SHV-11}$ was missed 25 times in strain EURGen-2022-02, *bla*$_{SHV-28}$ was missed 13 times in strain EURGen-2022-03 and *bla*$_{SHV-12}$ was missed three times in strain EURGen-2022-02 and two times in strain EURGen-2022-04), the *bla*$_{OXA}$ family (n=25 times, i.e., *bla*$_{OXA-9}$ was missed 15 times in strain EURGen-2022-03, *bla*$_{OXA-436}$ was missed six time in strain EURGen-2022-04, *bla*$_{OXA-1}$ was missed one time in strain EURGen-2022-02 and two times in strain EURGen-2022-03, and *bla*$_{OXA-10}$ was missed one time in strain EURGen-2022-04), and the *bla*$_{TEM}$ and *bla*$_{CTX-M}$ families (n=13 and n=2, respectively). The following most commonly missed genes encoded resistance towards aminoglycosides. In specific, genes belonging to the *aac(3)* family (n=31 times, i.e., *aac(3)-IIa* was missed 15 times in strain EURGen-2022-02 and 16 times in strain EURGen-2022-03), and the *acc(6')* family (n=14 times, i.e., *aac(6')-IIc* was missed six times in strain EURGen-2022-04, and *aac(6')-Ib-cr* was missed four times in strain EURGen-2022-02 and four times in strain EURGen-2022-03). The last category with a large number of missing genes were those encoding resistance to sulfonamides, specifically genes belonging to the *sul* family (n=28 times, i.e., *sul1* was missed seven times in strain EURGen-2022-02 and missed eight times in strain EURGen-2022-04, and *sul2* was missed five times in strain EURGen-2022-03 and missed eight times in strain EURGen-2022-04).

An important observation is that many of the missing genes encoding β-lactamases and mediating resistance towards aminoglycosides were closely represented within the unexpected genetic determinants of AMR reported by the participants. This means that although the specific expected gene variant was not correctly reported, a similar gene from the same class was reported instead. A notable example observed during this 2022 EQA was the reporting incorrect *bla*$_{SHV}$ genes with a high genetic similarity to the expected *bla*$_{SHV}$ variant (with, generally, over 99% of similarity between expected and incorrectly reported genes). Another explanation for reporting different but closely related genes is the heterogeneous naming and/or choice of reference sequences in bioinformatics databases, illustrated by the reporting of *aac(3)-IIe* instead of the expected *aac(3)-IIa* (the same genetic sequence is labeled differently in the AMRFinderPlus and ResFinder

databases) and by the reporting of *aac(6')-Ib* instead of the expected *aac(6')-IIc* (the same genetic sequence is labeled differently in the CARD-RGI and ResFinder databases). This is especially likely to occur if genes are recently published and there has been no harmonization of nomenclature yet. Solutions could be to ensure communication between curators of the most widely used databases, and to opt to use sequences that are part of reference sequence databases such as NCBI RefSeq (although this delays the updating of bioinformatics databases).

These problems suggest that a better harmonization between bioinformatics tools and their respective databases is needed, to ensure that the same genetic sequences have i) the same designation across databases and ii) the same potential for being detected across tools. Another solution could be to adopt a more general strategy for reporting AMR genes that allows for the acceptance of different variants. This would require an in-depth revision of bioinformatics tools' databases to avoid redundancy within the database, but also a careful curation of the same databases to ensure that special situations of similar variants that have different impacts in antimicrobial susceptibility are properly captured. Moreover, these findings support that the proper recording of bioinformatics tools, their respective versions and date of analysis are of paramount importance to allow for validation, traceability, and comparison of results within and between settings. During this EQA, it was observed that some participants did not report their approaches with enough granularity to allow this type of retroactive investigation (Appendices 1, 2 and 3), and they are encouraged to improve their data and metadata registry and reporting processes.

It was also observed that, in strains EURGen-2022-02 and EURGen-2022-03, many participants only reported one *bla*$_{SHV}$ or one *bla*$_{OXA}$ variant, respectively, instead of the expected two variants of those families. It is suspected that the presence of two closely related gene variants in the same genome created confusion or suspicion by the participants, leading to the report of only one of the variants. Participants are encouraged to confirm the genomic location of the relevant genetic determinants when analysing sequence data, including flanking regions, to confirm if different variants are present simultaneously or if this is an artifact of the bioinformatics tools (as exemplified below regarding different *bla*$_{TEM}$ variants detected in strain EURGen-2022-03). This confirmation is especially important when different variants from the same family are associated with different expected phenotypes (as discussed in the following section regarding different *bla*$_{OXA}$ variants and associated phenotypes in strain EURGen-2022-04).

Chromosomal PMs mediating decreased susceptibility towards quinolones were also frequently absent from reported results. The PM *parC* S80I was missing from 23 results (12 times in strain EURGen-2022-02 and 11 times in strain EURGen-2022-03) and the PM *gyrA* S83I was missing from 16 results (six times in strain EURGen-2022-02, four times in strain EURGen-2022-03 and six times in strain EURGen-2022-04). Participants should keep in mind that chromosomal PMs may have a cumulative effect (as it is the case for those mediating fluoroquinolone resistance), thus it is especially important to obtain a complete profile of these mutations.

Additionally, in some of the situations described previously, and others, there were presumable spelling, distraction or submission mistakes, such as selection of *mdt(A)* instead of *mdf(A)* for strain EURGen-2022-01 and *fomA* instead of *fosA* for strain EURGen-2022-02 (nevertheless not a part of expected results). There were also situations where PMs in *parC* and *gyrA* seemingly intended to be reported, but participants never defined the specific mutation to report, only the target gene.

The problem of reporting unexpected genetic determinants of AMR was even more prevalent than missed expected determinants and was observed in 72.1% of the sets of submitted results. Some of these situations have been addressed above, and are explainable by the detection of genes closely related to expected genes, especially those from the *bla*$_{SHV}$ and *aac(3)* families. It was furthermore observed that, in strain EURGen-

2022-03, there was a very high number of unexpected *bla*$_{TEM}$ variants instead or in addition to the expected *bla*$_{TEM-1}$. Reporting a different variant of the *bla*$_{TEM}$ gene would constitute another situation of detecting a closely related gene variant. However, there were nine participants that reported, simultaneously, at least two different variants of *bla*$_{TEM}$, which should be taken into careful consideration, for example by observing that some bioinformatics tools will provide information regarding the position of the detected genes in the genome, such as ResFinder, which was the tool used by eight of those participants. The information about position of these genes in the genome should make it obvious that these results are an artifact of the bioinformatics tool, which does not choose between different potential variants with similar percentages of identity and coverage, instead outputting all closely-related matches from the database. Therefore, participants were expected to have selected one variant to be reported in this EQA. Although presence of different variants from the same family is not impossible (as illustrated by several of the strains in this EQA, harbouring different β-lactamases from the same family), a high number of genes from the same family should instigate a critical analysis of the bioinformatics results.

For the most part, the remaining unexpectedly reported genetic determinants appear to be due to misinterpretation of the protocol of the 2022 EQA and insufficient knowledge regarding the impact of certain genes of PMs in the expected resistance profiles of the species included in this EQA. The genes *fosA*, *oqxA* and *oqxB* were incorrectly reported in strains EURGen-2022-02 and EURGen-2022-03. Although those genes are present in the genomes of those strains, they are intrinsic in *K. pneumoniae*, thus they do not contribute to a decrease of susceptibility towards any of the antimicrobials included in the 2022 EQA (respectively fosfomycin and ciprofloxacin). As for the *mcr-9* gene, this was incorrectly reported in strain EURGen-2022-04. This gene is not intrinsic in *Enterobacterales*, but it does not increase isolates' minimum inhibitory concentrations enough to lead to their classification as clinically resistant. Similar situations were observed for chromosomal PMs in *gyrA*, *parC*, *pmrA* and *pmrB*, that albeit present are not currently proven to be associated with decreased susceptibility towards quinolones and colistin, respectively. Several mutations were also reported in the target genes *acrR*, *ramR* and 16S-*rrsB*, and in the same way these might have an unconfirmed impact of the AMR profiles of the isolates, or they are associated with resistance towards antimicrobials not included in this EQA (such as tetracycline). According to the EQA protocol " *(…) only resistance genes and chromosomal mutations that are mediating resistance to any of the antimicrobials included in this EQA should be submitted (…)* ", therefore, the described genes and PMs were not part of the expected results. It should be kept in mind that *fosA* genes are not intrinsic in *E. coli* isolates and will contribute to increased resistance towards fosfomycin in this species. It should also be kept in mind that *mcr*-genes other than *mcr-9* will contribute to increased resistance towards colistin in *Enterobacterales*.

These situations show that laboratories should not report results from bioinformatics analyses blindly, but instead become familiar with the underlying genetic mechanisms of resistance that are relevant for the different species analysed in their settings. Reporting all AMR genes and chromosomal PMs found in an isolate appears like an appealing solution to avoid missing genetic determinants that might contribute towards AMR, but this might affect the choice of correct antibiotic therapy and lead to overuse of critically important last-line antimicrobials and promote the emergence or spread of resistance towards those antimicrobials. Furthermore, very important information can become more difficult to retrieve in the midst of very large datasets of results, and make it challenging to reach clinically and epidemiologically relevant conclusions.

4.5. *In silico* prediction of antimicrobial resistance profiles

The major discrepancies observed between expected and submitted results for the *in silico* prediction of AMR profiles were the lack of reporting predicted resistance towards ceftazidime-avibactam (n=43, in strains EURGen-2022-01 and EURGen-2022-02), and the lack of reporting predicted resistance towards carbapenems (and amoxicillin-clavulanic acid) in strain EURGen-2022-04. Furthermore, there was incorrect prediction of resistance towards fosfomycin (strains EURGen-2022-02 and EURGen-2022-03) and colistin (strains EURGen-2022-02 and EURGen-2022-04).

The absence of ceftazidime-avibactam, carbapenems and amoxicillin-clavulanic acid from several results illustrates the need for laboratories to become familiar with underlying genetic mechanisms of resistance so that they can critically evaluate results from bioinformatics analyses and avoid "false-negatives". The antimicrobial combination ceftazidime-avibactam is present in the most commonly used tool for prediction of AMR profiles, which was ResFinder; however, in the database of that tool, resistance towards ceftazidime-avibactam is not part of the output associated with the *bla*$_{\text{NDM-1}}$ gene (which is an error and exponentially increased the difficulty of this prediction, especially because the antimicrobial exists in the database associated with other genes). Similarly, the gene *bla*$_{\text{OXA-436}}$ exists in that database, but its phenotype does not include amoxicillin-clavulanic acid and carbapenems (which is, again, a limitation of the database). These problems support the need for laboratories to supplement their analysis with other bioinformatics tools and/or literature research, at least during the initial stages of implementation of WGS-based data analysis in their settings. Naturally, laboratories should also be familiar with the databases themselves in order to know if certain gene families or antimicrobial agents are not at all present. Additionally, these results once again show the importance of confirming the presence of different variants of the same genetic family, given the difference of expected phenotype for strain EURGen-2022-04 when considering *bla*$_{\text{OXA-436}}$, besides *bla*$_{\text{OXA-10}}$. Moreover, they highlight the importance of participating in international genomic EQAs, since analysis of data from these exercises reveals these specific problems and allows or the benchmarking of the different bioinformatics pipelines used in different settings.

The incorrect reporting of resistance towards fosfomycin and colistin, as discussed previously in relation to the detection of genetic determinants of AMR, are direct consequences of detection of *fosA* gene, *mcr-9* gene, and PMs in the gene *pmrB* not proven to be associated with decreased susceptibility towards colistin. Therefore, neither the genetic determinants nor the AMR profiles should be part of submitted results.

Finally, no participant predicted resistance of strain EURGen-2022-03 towards colistin, which was mediated by the optionally-reported chromosomal PM *mgrB::*IS1. This mutation (and thus, the respective associated phenotype) is not part of the ResFinder database, again defending the approach of using a confirmatory bioinformatics tool and the need to become familiar with the genetic mechanisms mediating AMR in different species, and their respective presence or absence in the chosen bioinformatics tools.

4.6. Addressing the feedback from the participants

The feedback from the participants was used to implement updates to the second and third EURGen-RefLabCap EQAs, to update the individual evaluation reports, to update this present aggregated report and to produce certificates of participation:

- The main change implemented to the second and third EQAs is the differentiated scoring of the AMR profiles for each antimicrobial, instead of scoring it as a complete profile for each strain;

- The main change implemented to the individual evaluation reports is a brief explanation of the context of the EQA, and an explanation of the empty scoring for certain situations that were manually adjusted;

- The main change implemented to this present report is the manual analysis and scoring of the individual AMR profiles, as well as clarification of the context of the EQA.

5. CONCLUSION AND RECOMMENDATIONS

The results from the EURGen-RefLabCap 2022 EQA show that, throughout Europe, there is still a lack of uniformity regarding analysis of WGS data for public health purposes such as clinical diagnostics and epidemiological surveillance.

Some of the discrepancies observed between expected and reported results appeared to be due to variations between the type of data and the chosen bioinformatics tools and databases. These should not be interpreted as a lack of capacity by the participants, but instead as indicators that further harmonization of bioinformatics approaches must be achieved internationally. Some actions that could improve comparability of results obtained in different settings are:

- Curators of bioinformatics tools and databases should engage in ongoing, active dialogue to ensure conformity between approaches;
- 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.

Other discordances detected during this EQA were due to misinterpretation of the EQA protocol and/or insufficient knowledge about certain genetic elements, leading to the reporting of unexpected plasmid replicons, AMR genes and chromosomal PMs. Furthermore, there were cases where important elements present in the data were not reported by the participants, and when resistance towards certain antimicrobials was not predicted. These issues appeared to be laboratory-specific and in some cases also appeared associated with the use of specific bioinformatics approaches. To increase local capacity, the proposed actions are:

- Laboratories should ensure sufficient knowledge about the genetic mechanisms mediating AMR and other important genetic elements;
- Laboratories should be familiar with 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.

Continued participation in genomic EQAs, the use of well-defined quality control parameters and respective thresholds, and the use of benchmarking datasets to validate different bioinformatics approaches are strategies that further contribute to the increase of local, national, and European capacity for WGS-based analysis and surveillance of important healthcare-associated pathogens. Participants of the EURGen-RefLabCap 2022 EQA who did not obtain results in full agreement with expected results are invited to repeat the analyses with the bioinformatics approaches and thresholds used to generate the expected results (as described in the "Methods" section of this report), and are welcome to contact the EQA organizers for support in troubleshooting in case they do not obtain the full set of expected results upon re-analyses.

6. APPENDICES

6.1. Appendix 1: Methods reported by the participants for prediction of MLST

Laboratory	Pipeline type	Software	Database	Parameters of the software	URL of the software or database
EURGen-RLC-001	Web-based	MLST 2.0.9	PubMLST	Default	https://cge.food.dtu.dk/services/MLST/
EURGen-RLC-002	Local	staramr v.0.7.1	staramr v.0.7.1	Default	https://github.com/phac-nml/staramr
EURGen-RLC-003	Web-based	MLST 2.0.9 (2022-05-11)	Database version: (2022-10-24) ¤ Escherichia coli #1; Klebsiella pneumoniae	Default	https://cge.food.dtu.dk/services/MLST/ ¤ MLST allele sequence and profile data is obtained from PubMLST.org
EURGen-RLC-004	Local	publicly available software: mlst 2.19.0	Database: PubMLST	Default	https://github.com/tseemann/mlst
EURGen-RLC-005	Web-based	MLST 2.0	PubMLST.org	Default	https://cge.food.dtu.dk/services/MLST/
EURGen-RLC-007	Web-based	pubmlst.org/mlst	pubmlst.org/mlst	Default	pubmlst.org/mlst
EURGen-RLC-008	NA	NA	CGE - MLST 2.0	NA	NA
EURGen-RLC-009	Local	Ridom SeqSphere+	Ridom SeqSphere+ database updated daily from pubMLST	NA	https://www.ridom.de/seqsphere/
EURGen-RLC-010	Web-based	MLST version: 2.0.9	Database version: (2022-10-31)	Default	MLST 2.0 (dtu.dk)
EURGen-RLC-011	Local	srst2	publicly available	Default	https://github.com/katholt/srst2
EURGen-RLC-012	Local	MLST finder	publicly available	default	NA
EURGen-RLC-014	Local	commercial software, Ridom SeqSphere+ version 8.3.4	publicly available database; E. coli MLST Warwick version 1.0, K. pneumoniae MLST Institute Pasteur version 1.0; analysis via Ridom SeqSphere+ version 8.3.4	Default	E. coli MLST: enterobase.warwick.ac.uk/species/index/ecoli ¤ K. pneumoniae MLST: bigsdb.pasteur.fr/klebsiella
EURGen-RLC-015	Web-based	Publicly available software	Publicly available database	Default	https://cge.food.dtu.dk/services/ResFinder/ ¤ https://github.com/phac-nml/staramr
EURGen-RLC-016	Web-based	NA	publicly available database (MLST 2.0)	Default	https://cge.food.dtu.dk/services/MLST/
EURGen-RLC-017	NA	BioNumerics 8.1	NA	NA	NA
EURGen-RLC-018	Web-based	MLST 2.0, Software version: 2.0.9 (2022-05-11)	PubMLST.org. Database version: (2022-10-24)	Default	https://cge.food.dtu.dk/services/MLST/
EURGen-RLC-019	Local	public, mlst, 2.19.0	public, Pubmlst, 2.19.0	Default	https://github.com/tseemann/mlst

Laboratory	Pipeline type	Software	Database	Parameters of the software	URL of the software or database
EURGen-RLC-020	Local	in-house script using program mlst	Publicly available database - PubMLST (last updated 2022-10-06)	Default	Program: https://github.com/tseemann/mlst ¤ Database: https://pubmlst.org/
EURGen-RLC-021	Local	Ridom Seqsphere 8.4.1	NA	Default	NA
EURGen-RLC-022	Web-based	Publicly available software - CGE MLST (V 2.0.9)	Publicly Available Database - Database Version (2022-10-24)	Default	https://cge.food.dtu.dk/services/MLST/
EURGen-RLC-023	Local	https://github.com/tseemann/mlst v.2.21.0	https://github.com/tseemann/mlst v.2.21.0	Default	https://github.com/tseemann/mlst v.2.21.0
EURGen-RLC-024	Web-based	Center for Genomic Epidemiology, MLST - publicly available software, version 2.0.9;	Center for Genomic Epidemiology, MLST publicly available database; database version 24.10.2022	Default	https://cge.food.dtu.dk/services/MLST/
EURGen-RLC-026	Web-based	publicly available software/ MLST 2.0.9	publicly available database/ (2022-10-24)	Default	https://cge.food.dtu.dk/services/MLST/
EURGen-RLC-027	Local	Commercial Software - Ridom SeqSphere+ version 8.4.4.2	Public available database - PubMLST	Default	https://pubmlst.org/
EURGen-RLC-028	Web-based	MLST 2.0 (Center for Genomic Epidemiology)	Database version 2022-10-11	MLST Configuration Escherichia coli#1, Klebsiella pneumoniae ¤ Min. depth for an allele 10x	https://cge.food.dtu.dk/services/MLST/
EURGen-RLC-029	Local	Ridom SeqSphere+ v8.4.1	Warwick(E.c)/Institute Pasteur (K.p)	Default	na
EURGen-RLC-030	NA	Center for Genomic Epidemiology, MLST 2.0, v. 2.0.9	version 2022-10-24	Default	https://cge.food.dtu.dk/services/MLST/
EURGen-RLC-031	Local and web-based	RidomSeqSphere and MLSTFinder	Publicly available database (PubMLST.org 2022-10-24)	Default	https://www.ridom.de/ ¤ https://cge.food.dtu.dk/services/MLST/
EURGen-RLC-032	Local	Mix of commercial and in-house	in-house built on publicaly avilable databases	Pass = 10X depth and 100% coverage	N/A

6.2. Appendix 2: Methods reported by the participants for detection of plasmid replicons

Laboratory	Pipeline type	Software	Database	Parameters of the software	URL of the software or database
EURGen-RLC-001	Web-based	PlasmidFinder 2.0.1	2021-11-29	Default	https://cge.food.dtu.dk/services/PlasmidFinder-2.0/
EURGen-RLC-002	Local	staramr v.0.7.1	staramr v.0.7.1	Default	https://github.com/phac-nml/staramr
EURGen-RLC-003	Web-based	PlasmidFinder, Software version: 2.0.1 (2020-07-01)	Enterobacteriales Database version: (2021-11-29)	minimum length 80% and minimum identity 95%	https://cge.food.dtu.dk/services/PlasmidFinder/
EURGen-RLC-004	Local	publicly available software: Abricate 1.0.1 ¤	publicly available database: plasmidfinder_db (2021-11-29)	'abricate {file} --threads 64 --nopath --db plasmidfinder > {output_file}'	https://github.com/tseemann/abricate
EURGen-RLC-005	Web-based	PlasmidFinder 2.1.	plasmidfinder_db	Default	https://cge.food.dtu.dk/services/PlasmidFinder/
EURGen-RLC-007	Local	publicly available software, plasmidfinder (v. 2.1.6)	publicly available software, plasmidfinder (2.1.6)	Default	NA
EURGen-RLC-008	NA	NA	CGE - PlasmidFinder 2.1	NA	NA
EURGen-RLC-009	Local	In-house script (Bifrost)	PlasmidFinder 2.1	Minimum length 95% and minimum identity 90%	https://bitbucket.org/genomicepidemiology/plasmidfinder_db/src/master/
EURGen-RLC-010	Web-based	PlasmidFinder version: 2.0.1	Database version: (2021-11-29)	Default	PlasmidFinder 2.1 (dtu.dk)
EURGen-RLC-011	Local and web-based	CGE plasmid finder and FFPA-pipeline	CGE:n plasmid database in both	Default	etsi github sivu!
EURGen-RLC-012	Local	Plasmid Finder 2.1.8	publicly available	Default	NA
EURGen-RLC-014	Web-based	publicly available software, PlasmidFinder 2.1 (Center for Genomic Epidemiology) version 2.0.1 (2020-07-01)	publicly available database, PlasmidFinder database, Enterobacteriales, version 2021-11-29	Default	cge.food.dtu.dk/services/PlasmidFinder
EURGen-RLC-015	Web-based	Publicly available software	Publicly available database	Default	https://cge.food.dtu.dk/services/ResFinder/ ¤ https://github.com/phac-nml/staramr
EURGen-RLC-016	Web-based	NA	publicly available database (PlasmidFinder 2.1)	Default	https://cge.food.dtu.dk/services/PlasmidFinder/
EURGen-RLC-017	NA	BioNumerics 8.1, E.coli plug in 2.1	NA	NA	NA
EURGen-RLC-018	Web-based	PlasmidFinder 2.1, Software version: 2.0.1 (2020-07-01)	Database version: (2021-11-29), Test sequence	Default	https://cge.food.dtu.dk/services/PlasmidFinder/
EURGen-RLC-019	Local	public, Mob_suite, 3.0.3	public, Mob_suite, 2022-05-16	Default	https://github.com/phac-nml/mob-suite

Laboratory	Pipeline type	Software	Database	Parameters of the software	URL of the software or database
EURGen-RLC-020	Local	In-house script using PlasmidFinder	Publicly available databse - plasmidfinder_db (last updated 2022-10-06)	Default	PlasmidFinder: https://bitbucket.org/genomicepidemiology/plasmidfinder/src/master/ ¤ plasmidfinder_db: https://bitbucket.org/genomicepidemiology/plasmidfinder_db/src/master/
EURGen-RLC-021	Web-based	NA	PlasmidFinder 2.0	Default	https://cge.food.dtu.dk/services/PlasmidFinder/
EURGen-RLC-022	Web-based	Publicly available software - CGE PlasmidFinder (V 2.0.1)	Publicly Available Database - Database Version (2021-11-29)	Minimum Identity 95% and Minimum Length 80%	https://cge.food.dtu.dk/services/PlasmidFinder/
EURGen-RLC-023	Local	in-house PlasmidFinder v.2.0.1	PlasmidFinder 2021-11-29	minimum length 90%, minimum identity 90%	https://cge.cbs.dtu.dk/services/PlasmidFinder/
EURGen-RLC-024	Web-based	PlasmidFinder Software version: 2.0.1 (2020-07-01) ¤ MobileElementFinder, software version v1.0.3 (2020-10-09)	PlasmidFinder Database version: (2021-11-29) ¤ MobileElementFinder Database version: v1.0.2 (2020-06-09)	PlasmidFinder 2.1, web-based pipeline - default parameters, 95% identity, 60% coverage	https://cge.food.dtu.dk/services/PlasmidFinder/ ¤ https://cge.food.dtu.dk/services/MobileElementFinder/
EURGen-RLC-026	Web-based	publicly available software/ PlasmidFinder 2.0.1	(2021-11-29)	Default	https://cge.food.dtu.dk/services/PlasmidFinder/
EURGen-RLC-027	Web-based	publicly available software PlasmidFinder2.1	publicly available database	Default	https://cge.food.dtu.dk/services/PlasmidFinder/
EURGen-RLC-028	Web-based	PlasmidFinder 2.1 (Center for Genomic Epidemiology)	plasmidfinder_db ver.2021-11-29	Selected database: Enterobacteriales ¤ Select threshold for minimum % identity: 95% ¤ Select minimum % coverage: 60%	https://cge.food.dtu.dk/services/PlasmidFinder/
EURGen-RLC-029	Web-based	PlasmidFinder 2.1 (Center for Genomic Epidemiology)	PlasmidFinder2.1 (Database version: (2021-11-29))	Default	https://cge.food.dtu.dk/services/PlasmidFinder/
EURGen-RLC-030	NA	Center for Genomic Epidemiology, PlasmidFinder 2.1, v 2.0.1	version 2021-11-29	selected database: Enterobacteriales ¤ everything else default parameters	https://cge.food.dtu.dk/services/PlasmidFinder/
EURGen-RLC-031	Local and web-based	PlasmidID, PlasmidFinder and MGE	Database version for PlasmidFinder 2021-11-29 ¤ Database for plasmidid : https://github.com/BU-ISCIII/plasmidID/wiki/Plasmid-Database	Minimum length 80% and minimum identity 98%	https://github.com/BU-ISCIII/plasmidID

Laboratory	Pipeline type	Software	Database	Parameters of the software	URL of the software or database
EURGen-RLC-032	Local	Mix of commercial and in-house	in-house built on publicaly avilable databases	Pass = 10X depth and 100% coverage	N/A

6.3. Appendix 3: Methods reported by the participants for detection of genetic determinants of AMR and prediction of AMR profiles

Laboratory	Pipeline type	Software	Database	Parameters of the software	URL of the software or database
EURGen-RLC-001	Local and web-based	ResFinder 4.1; AMRFinderPlus software v3.10.5	EFSA_2021 (2022-05-24), AMRFinderPlus db v2021-06-01.1, PointFinder database (2021-02-01)	Default	https://cge.food.dtu.dk/services/ResFinder-4.1/
EURGen-RLC-002	Local	staramr v.0.7.1; ARIBA; ncbi-amrfinderplus_v3.1.30	staramr v.0.7.1/CNR version (ARM(100)|VIR(38)) (French NRC)/2022-05-26.1	Default	https://github.com/phac-nml/staramr/NA/https://github.com/ncbi/amr
EURGen-RLC-003	Web-based	ResFinder 4.1 and PointFinder software: (2022-08-08)	ResFinder database: EFSA_2021 (2022-05-24) ¤ PointFinder database: (2021-02-01)	minimum length 60% and minimum identity 90%.	https://cge.food.dtu.dk/services/ResFinder/
EURGen-RLC-004	Local and web-based	publicly available software: AMRFinderPlus v3.10.40 / publicly available software: ResFinder 4.1 and PointFinder / publicly available software: Abricate 1.0.1	Database: NCBI ¤ Database version: 2022-10-11.2 ¤ ResFinder database: EFSA_2021 (2022-05-24) ¤ PointFinder database: (2021-02-01) ¤	AMRFinderPlus v3.10.40; ResFinder 4.1: default; Abricate 1.0.1	https://github.com/ncbi/amr ¤ https://cge.food.dtu.dk/services/ResFinder/ ¤ https://github.com/tseemann/abricate
EURGen-RLC-005	Web-based	ResFinder 4.1	ResFinder and PointFinder database	Default	https://cge.food.dtu.dk/services/ResFinder/
EURGen-RLC-007	Local and web-based	publicly available software, AMRFinderPlus (v. 3.8.4) / publicly available, ResFinder (v. 4.1.)	publicly available database,	Default	https://cge.food.dtu.dk/resfinder
EURGen-RLC-008	NA	NA	CGE - ResFinder 4.1	NA	NA
EURGen-RLC-009	Local and web-based	In-house script (Bifrost) / ResFinder 4.1	ResFinder 4.1	Default / Minimum length 95% and minimum identity 90%	https://bitbucket.org/genomicepidemiology/resfinder_db/src/master/
EURGen-RLC-010	Web-based	ResFinder 4.1	ResFinder database: (2022-05-24); PointFinder database: (2022-04-22)	Default	ResFinder 4.1 (dtu.dk)
EURGen-RLC-011	Local and web-based	srst2 and CGE ResFinder	CARD version 3.0.8	Default	https://github.com/katholt/srst2
EURGen-RLC-012	Local	in house scripts, in house tool "AMR seq detetor"	in house database : ARM database, v100	Do not know	NA
EURGen-RLC-014	Local and web-based	commercial software, Ridom SeqSphere+ version 8.3.4 / publicly	publicly available database, NCBI AMRFinderPlus 1.1, , ResFinder 4.1	Default	ncbi.nlm.nih.gov/pathogens/antimicrobial-resistance/AMRFinder /

		available software, ResFinder 4.1			https://cge.food.dtu.dk/services/ResFinder/
EURGen-RLC-015	Web-based	Publicly available software	Publicly available database	Default	https://cge.food.dtu.dk/services/ResFinder/ ¤ https://github.com/phac-nml/staramr
EURGen-RLC-016	Web-based	NA	publicly available database (ResFinder 4.1, RGI 6.0.0, CARD 3.2.5)	Default	https://cge.food.dtu.dk/services/ResFinder/, https://card.mcmaster.ca/analyze/rgi
EURGen-RLC-017	Web-based	BioNumerics 8.1, E.coli plug in 2.1	NA	Default	NA
EURGen-RLC-018	Web-based	ResFinder 4.1, ResFinder and PointFinder software: (2022-08-08)	ResFinder database: EFSA_2021 (2022-05-24), PointFinder database: (2021-02-01)	Default	https://cge.food.dtu.dk/services/ResFinder/
EURGen-RLC-019	Local	public, Resfinder, 4.1.11 / public, rgi, 5.2.1	public, resfinder_db, 2022-05-11, public, card, 3.1.4	Default	https://bitbucket.org/genomicepidemiology/resfinder/src/master/ / https://github.com/arpcard/rgi
EURGen-RLC-020	Local	In-house script that uses AMRFinderPlus program	Publicly available database derived from the Pathogen Detection Reference Gene Catalog, Pathogen Detection Reference Gene Hierarchy, and Reference HMM Catalog. Last update 2022-10-12	Default	AMRFinderPlus https://github.com/ncbi/amr ¤ Database https://ftp.ncbi.nlm.nih.gov/pathogen/Antimicrobial_resistance/AMRFinderPlus/database/latest/
EURGen-RLC-021	Web-based	NA	ResFinder 4.1	Default / Minimum length 100% and minimum identity 100%	https://cge.food.dtu.dk/services/ResFinder/
EURGen-RLC-022	Web-based	Publicly available software - CGE ResFinder (V 4.1)	Publicly Available Database - Database Version EFSA_2021 (2022-05-24)	Minimum Identity 95% and Minimum Length 80%	https://cge.food.dtu.dk/services/ResFinder/
EURGen-RLC-023	Local	AmrFinderPlus v.3.10.16	Database v.2022-08-09.1	minimum length 90% and minimum identity 90% and minimum length 60% and minimum identity 60%	https://www.ncbi.nlm.nih.gov/pathogens/antimicrobial-resistance/AMRFinder/
EURGen-RLC-024	Web-based	ResFinder and PointFinder software: (2022-08-08)	ResFinder database: EFSA_2021 (2022-05-24) ¤ PointFinder database: (2021-02-01)	ResFinder 4.1, default parameters, 90% ID, 60% minimum length	https://cge.food.dtu.dk/services/ResFinder/

EURGen-RLC-026	Web-based	publicly available software/ ResFinder 4.1	publicly available database / EFSA_2021 (2022-05-24)	Default	https://cge.food.dtu.dk/services/ResFinder/
EURGen-RLC-027	Local and web-based	AMRFinderPlus included in Ridom SeqSphere+ ¤ ResFinder 4.1 / BLAST	publicly available database	Default	https://cge.food.dtu.dk/services/ResFinder/
EURGen-RLC-028	Web-based	ResFinder-4.1	resfinder_db EFSA_2021 2022-05-24, pointfinder_db 2021-02-01	Select threshold for %ID: 90%, Select minimum length: 60%, Show unknown mutations, not found in the database: Yes	https://cge.food.dtu.dk/services/ResFinder/
EURGen-RLC-029	Local and web-based	KmerResistance 2.2 , ResFinder 4.1 , ABRICATE 1.0.1	KmerResistance 2.2 / ResFinder and PointFinder software: (2022-08-08) / ResFinder 2021-mar-27	Default / minimum length 100% and minimum identity 98%	https://cge.food.dtu.dk/services/KmerResistance/ / https://cge.food.dtu.dk/services/ResFinder/
EURGen-RLC-030	NA	ResFinder 4.1; MGE v1.0.3 (202-10-09)	version EFSA_2021 (2022-05-24); ¤ version v1.0.2 (202-06-09)	Default	https://cge.food.dtu.dk/services/ResFinder/ ¤ https://cge.food.dtu.dk/services/MobileElementFinder/
EURGen-RLC-031	Local and web-based	Resfinder, ARIBA (with Resfinder, Megares and Card databases) / RidomSeqsphere under personal expertice.	Resfinder EFSA_2021(2022-05-24) ¤ Megares 3.0.0 ¤ Card 3.2.5	Minimum length 80% and minimum identity of 98% except for b-lactamases that we applied 99%.	https://github.com/sanger-pathogens/ariba / https://cge.food.dtu.dk/services/ResFinder/ / https://www.ridom.de/
EURGen-RLC-032	Local	Mix of commercial and in-house	in-house built on publicaly avilable databases	Pass = 10X depth and 100% coverage	N/A

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