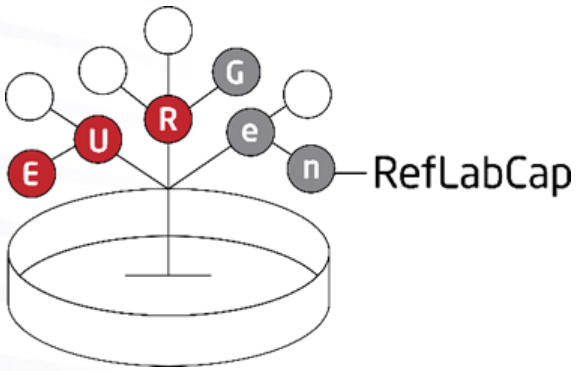
European Commission

EURGen-RefLabCap

Report from the second external quality assessment exercise



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European Health and Digital Executive Agency

Third EU Health Programme

Report from the second external quality assessment exercise

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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 CRE (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 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 using 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 order of increasing challenge (Figure 1). The first EQA, conducted in 2022, included WS1 pathogens and encompassed analysis of WGS data using 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, and the focus of this current report, also included 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, will include the handling of live bacterial isolates and DNA extraction, to also assess local capacity for those steps of the sequencing process.

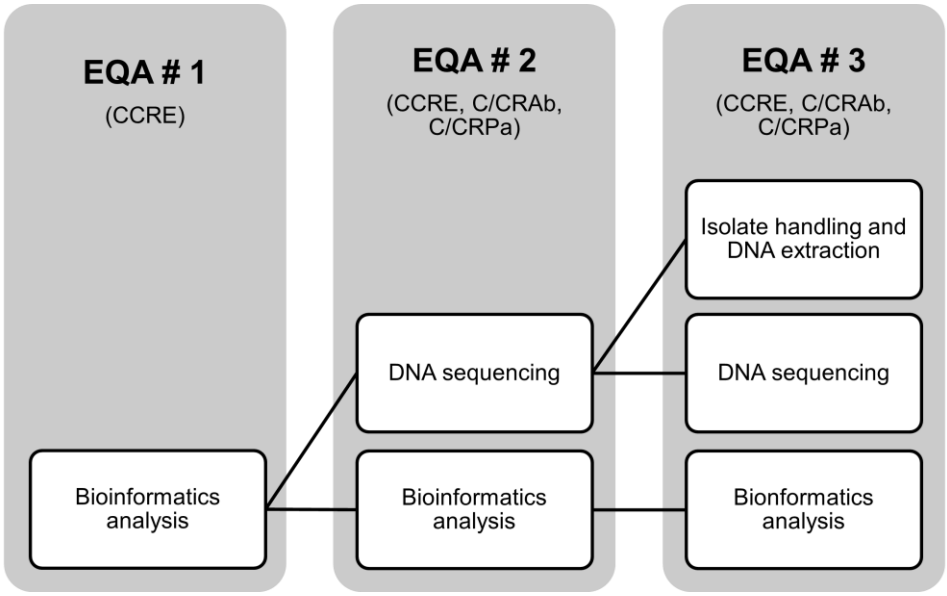

Figure 1. Representation of the three EQAs planned in the EURGen-RefLabCap project

The second EURGen-RefLabCap EQA included pure vacuum-dried DNA samples of four strains (one *Escherichia coli*, one *Klebsiella pneumoniae*, one *A. baumannii* and one *P. aeruginosa* strain). The laboratories nominated for WS1 of the EURGen-RefLabCap project could submit results for the *E. coli* and *K. pneumoniae* strains, and the laboratories nominated for WS2 of the project could submit results for the *A. baumannii* and *P. aeruginosa* strains. Laboratories nominated for both workstreams could submit results for all four strains. The EQA included: i) DNA sequencing with any desired technology; ii) prediction of sequence type (ST) based on multilocus sequence typing (MLST); iii) detection of plasmid replicon types; iv) detection of genes and chromosomal point mutations (PMs) mediating antimicrobial resistance (AMR), and; v) *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. The number of laboratories that signed up for the EQA exercise was 31 while 30 laboratories submitted their results. Of these, two laboratories submitted results for WS1 pathogens, and 28 laboratories submitted results for all pathogens.

2. EXERCISE DESIGN AND METHODS

2.1. EQA material

The material for the EURGen-RefLabCap 2023 EQA corresponded to purified DNA obtained from one *A. baumannii* (EURGen-2023-01), one *E. coli* (EURGen-2023-02), one *K. pneumoniae* (EURGen-2023-03), and one *P. aeruginosa* (EURGen-2023-04) strain. These strains were selected based on their genomic content, including ST, plasmid replicons and genetic determinants associated with resistance towards carbapenems, colistin and other antimicrobials of clinical relevance. For each strain, participants received one Eppendorf® tube containing at least 125 ng of vacuum-dried DNA. In case participants were unable to sequence the DNA, sequence data was available upon request: either assembled data files (FASTA files) or raw sequence data files (FASTQ files), produced either with short-read sequencing technologies (Illumina, Inc., San Diego, CA, United States of America) or with long-read sequencing technologies (Oxford Nanopore Technologies, Inc., Oxford, United Kingdom).

Table 1. Overview of material available to the participants for the EURGen-RefLabCap 2023 EQA

Strain	Material code	Description
EURGen-2023-01 (*A. baumannii*)	EURGen-2023-01	Approximately 125 ng of pure, vacuum-dried DNA
	EURGen-2023-01_Illumina.fasta	Assembled reads produced with short-read sequencing
	EURGen-2023-01_Nanopore.fasta	Assembled reads produced with long-read sequencing
	EURGen-2023-01_Illumina_R1.fastq EURGen-2023-01_Illumina_R2.fastq	Raw data files produced with short-read sequencing
	EURGen-2023-01_Nanopore.fastq	Raw data file produced with long-read sequencing
EURGen-2023-02 (*E. coli*)	EURGen-2023-02	Approximately 125 ng of pure, vacuum-dried DNA
	EURGen-2023-02_Illumina.fasta	Assembled reads produced with short-read sequencing
	EURGen-2023-02_Nanopore.fasta	Assembled reads produced with long-read sequencing
	EURGen-2023-02_Illumina_R1.fastq EURGen-2023-02_Illumina_R2.fastq	Raw data files produced with short-read sequencing
	EURGen-2023-02_Nanopore.fastq	Raw data file produced with long-read sequencing
EURGen-2023-03 (*K. pneumoniae*)	EURGen-2023-03	Approximately 125 ng of pure, vacuum-dried DNA
	EURGen-2023-03_Illumina.fasta	Assembled reads produced with short-read sequencing
	EURGen-2023-03_Nanopore.fasta	Assembled reads produced with long-read sequencing
	EURGen-2023-03_Illumina_R1.fastq EURGen-2023-03_Illumina_R2.fastq	Raw data files produced with short-read sequencing
	EURGen-2023-03_Nanopore.fastq	Raw data file produced with long-read sequencing
EURGen-2023-04 (*P. aeruginosa*)	EURGen-2023-04	Approximately 125 ng of pure, vacuum-dried DNA
	EURGen-2023-04_Illumina.fasta	Assembled reads produced with short-read sequencing
	EURGen-2023-04_Nanopore.fasta	Assembled reads produced with long-read sequencing

Strain	Material code	Description
	EURGen-2023-04_Illumina_R1.fastq EURGen-2023-04_Illumina_R2.fastq	Raw data files produced with short-read sequencing
	EURGen-2023-04_Nanopore.fastq	Raw data file produced with long-read sequencing

The DNA samples were prepared at DTU with Invitrogen™ Easy-DNA™ gDNA Purification kit (Thermo-Fischer Scientific, Massachusetts, United States). The short-read sequencing data were obtained at DTU using Illumina NextSeq™ 500 (Illumina, Inc., San Diego, CA, United States of America). The libraries were prepared with Illumina Nextera™ XT DNA Library Preparation Kit – 96 Samples using on Illumina Nextera™ XT DNA library preparation reference guide (Version 06, August 2021). Long-read sequencing data were obtained with GridION™ (Oxford Nanopore Technologies, Inc., Oxford, United Kingdom) using R10.4.1 flow cells (FLO-MIN114). The libraries were prepared with Rapid Barcoding Kit 96 V14, (SQK-RBK114.96) (Oxford Nanopore Technologies, Inc., Oxford, United Kingdom) using the Rapid sequencing DNA V14 – barcoding protocol (SQK-RBK114.96, VERSION: RBK_9176_V114_REVA_27NOV2022). The quality control of raw short reads was performed using FastQC[1] v0.11.5 and quality trimming was performed using BBDuK2 v36.49[2]. The quality control of raw long-reads was evaluated using NanoStat[3] 1.4.0 and NanoPlot[4] v1.41.6 and trimming was performed using Filtong[5] v.02.1. The genome assembly from short-reads was performed using SPAdes Genome Assembler[6] v3.11.0, while genome assembly from long-reads was performed with Flye v1.0[7]. Species verification was conducted with command-line KmerFinder[8] v3.2, database version 2022-07-11.

2.2. Expected results

The expected bioinformatics analysis results were produced at DTU and SSI. At DTU, the expected results were produced using a suite of bioinformatics tools and databases including the tools available at the Center for Genomic Epidemiology (CGE), using the short-read and long-read sequencing data files (FASTA and FASTQ):

- STs were predicted with command-line MLST[9] v2.0.9, database version 2023-04-24, using the schemes "*Acinetobacter baumannii* #1", "*Escherichia coli* #1" "*Klebsiella pneumoniae*", and "*Pseudomonas aeruginosa*";
- Plasmid replicons were detected with command-line PlasmidFinder[10] v2.0.1 (2020-07-01), database version 2023-01-18 with minimum thresholds of identity: 90% and coverage: 90%;
- AMR genes and chromosomal mutations conferring AMR were detected with command-line ResFinder[11] v4.1, ResFinder database version 2022-07-19 and

[1] https://github.com/s-andrews/FastQC
[2] https://github.com/BioInfoTools/BBMap
[3] https://github.com/wdecoster/nanostat
[4] https://github.com/wdecoster/NanoPlot
[5] https://github.com/rrwick/Filtlong
[6] https://github.com/ablab/spades
[7] https://github.com/fenderglass/Flye
[8] https://cge.food.dtu.dk/services/KmerFinder/
[9] https://cge.food.dtu.dk/services/MLST/
[10] https://cge.food.dtu.dk/services/PlasmidFinder/
[11] https://cge.food.dtu.dk/services/ResFinder/

PointFinder database version 2022-04-22, with minimum thresholds of identity: 80% and coverage: 60%. Additionally, command-line AMRFinderPlus[12] v3.11.17 and Resistance Genes Identifier (RGI)[13] with CARD database v3.2.6 were also used to detect AMR genes and mutations.

To generate the expected results at SSI, the DNA extracted at DTU was sequenced with both short-read and long-read sequencing technology. The short-read sequencing data at SSI were obtained using Illumina NextSeq™ 550 (Illumina, Inc., San Diego, CA, United States of America) using the Illumina Nextera™ XT DNA Library Preparation Kit following the Illumina Nextera™ XT DNA library preparation reference guide (Version 06, August 2021). The long-read sequencing was performed with GridION™ (Oxford Nanopore Technologies, Inc., Oxford, United Kingdom) and R10.4.1 flow cells (FLO-MIN114). The libraries were prepared with Rapid Barcoding Kit V14 using the Rapid sequencing DNA V14 barcoding protocol (SQK-RBK114.96, VERSION: RBK_9176_V114_REVA_27NOV2022). The quality control of raw reads, both for short- and long-read data, was performed using Bifrost QC and analysis pipeline[14]. The genome assembly from short-reads was performed using SKESA v2.4.0[15], while genome assembly from long-reads was performed with Flye v2.9.2[16]. The results regarding STs, plasmid replicons, genes and chromosomal mutations mediating AMR, and prediction of AMR profiles were obtained by using two methods in parallel, including:

- STs were predicted with MLST[17] v2.0.9, database version 2023-04-24 using CGE server, using the schemes "*Acinetobacter baumannii* #1", "*Escherichia coli* #1" "*Klebsiella pneumoniae*", and "*Pseudomonas aeruginosa*";

- Identification of plasmid replicons in all four test strains was performed using PathogenWatch with default parameters. Of note, PathogenWatch uses PlasmidFinder, and the PlasmidFinder database is designed to detect plasmids in *Enterobacterales*. Therefore, it is expected that, by using this method, no plasmid replicons are found in species not belonging to *Enterobacterales*. At present, repliocn typing of plasmids in *A. baumannii* and *P. aeruginosa* is not as standardised as in *Enterobacterales*;

- AMR genes and chromosomal mutations conferring antimicrobial resistance were detected using web-based ResFinder[18] v4.1, ResFinder database version 2022-07-19 and PointFinder database version 2022-04-22; PathogenWatch was used for detecting AMR genes and mutations in EURGen-2023-03 using default parameters.

The consensus expected results were produced by critically evaluating the outcome of the methods used by the two institutions and by 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 2, 3 and 4.

Table 2. Expected MLST results for the material included in the 2023 EQA

Material	**ST**	**Alleles assigned to each locus, from the Oxford scheme for *A. baumannii***

[12] https://github.com/ncbi/amr
[13] https://card.mcmaster.ca/analyze/rgi
[14] https://github.com/ssi-dk/bifrost
[15] https://github.com/ncbi/SKESA
[16] https://github.com/fenderglass/Flye
[17] https://cge.food.dtu.dk/services/MLST/
[18] https://cge.food.dtu.dk/services/ResFinder/

		cpn60	*gdhB*	*gltA*	*gpi*	*gyrB*	*recA*	*rpoD*
EURGen-2023-01	136[a]	2	3[a]	1	16	3	2	3
Material	**ST**	**Alleles assigned to each locus, from the Achtman scheme for *E. coli***						
		adk	*fumC*	*gyrB*	*icd*	*mdh*	*purA*	*recA*
EURGen-2023-02	410	6	4	12	1	20	18	7
Material	**ST**	**Alleles assigned to each locus, from the scheme *K. pneumoniae***						
		gapA	*infB*	*mdh*	*pgi*	*phoE*	*rpoB*	*tonB*
EURGen-2023-03	4568	2	1	2	1	247	1	46
Material	**ST**	**Alleles assigned to each locus, from the scheme *P. aeruginosa***						
		acsA	*aroE*	*guaA*	*mutL*	*nuoD*	*ppsA*	*trpE*
EURGen-2023-04	233	16	5	30	11	4	31	41

[a] The Oxford scheme reports two sequence types for EUGen-2023-01 due to presence of multicopy *gdhB* allele, specifically *gdhB*_189 and *gdhB*_3. The allele and ST with lowest number was selected for the expected results.

Table 3. Expected plasmid replicon results for the material included in the 2023 EQA

Material	**Plasmid replicons**
EURGen-2023-01	No plasmid replicon detected
EURGen-2023-02	**Expected:** Col(BS512), ColKP3, IncFIA, IncFIB(AP001918), IncFII(pAMA1167-NDM-5), IncX3
	Expected but non-mandatory: IncQ1, Col(pHAD28)
EURGen-2023-03	**Expected**: repB(R1701)
	Expected but non-mandatory: Col(pHAD28)
EURGen-2023-04	No plasmid replicon detected

Material	**AMR genes and chromosomal mutations**

EURGen-2023-01 (*A. baumannii*)	**Expected**: *aph(3')-VI*[a], *armA, bla*$_{NDM-1}$, *bla*$_{OXA-23}$, *gyrA* S81L, *parC* S84L
	Expected but non-mandatory: *ftsI* A515V, *parC* V104I, *parC* D105E
EURGen-2023-02 (*E. coli*)	**Expected:** *aac(3)-IId*, *aac(6')-Ib-cr*[b], *bla*$_{CMY-2}$[c], *bla*$_{OXA-181}$, *bla*$_{NDM-5}$, *bla*$_{TEM-1}$[d], *bla*$_{CTX-M-15}$, *qnrS1*, *sul1*, *sul2*, *dfrA12*, *dfrA17, glpT* E448K, *gyrA* D87N, *gyrA* S83L, *parE* S458A, *parC* S80I
	Expected but non-mandatory: *bla*$_{OXA-1}$, *ftsI* N337NYRIN, *pmrB* Y358N
EURGen-2023-03[e,f] (*K. pneumoniae*)	**Expected:** *bla*$_{SHV-1}$[e], *bla*$_{TEM-1}$[d], *bla*$_{CTX-M-3}$, *qnrS1*
	Expected but non-mandatory: *mgrB*::IS*1*
EURGen-2023-04 (*P. aeruginosa*)	**Expected:** aac(6')-Il, aac(3)-Id, *bla*$_{VIM-2}$, *bla*$_{OXA-4}$, *crpP, gyrA* T83I
	Expected but non-mandatory: *parC* S87L

Table 4. Expected acquired AMR genes and chromosomal PMs mediating AMR included in the 2023 EQA

[a] Either *aph(3')-VIa* or *aph(3')-VI*
[b] Either *aac(6')-Ib-cr5* or *aac(6')-Ib-cr*
[c] Either the *bla*$_{CMY-2}$ or *bla*$_{CMY-59}$
[d] Either *bla*$_{TEM-1}$, *bla*$_{TEM-1A}$, *bla*$_{TEM-1B}$, *bla*$_{TEM-1C}$ or *bla*$_{TEM-1D}$
[e] Either *bla*$_{SHV-1}$, *bla*$_{SHV-185}$ or *bla*$_{SHV-187}$.
[f] The recent findings suggest that blaSHV is intrinsic in *K. pneumoniae,* however in EQA 2023, it was considered as acquired AMR gene and was included in the expected results.

Table 5. Expected *in silico* prediction of AMR profiles for the material included in the 2023 EQA

Material	Associated prediction of AMR profiles
EURGen-2023-01 (*A. baumannii*)	**Expected**: Amikacin, ciprofloxacin, cefepime, ceftazidime, ceftazidime-avibactam, gentamicin, imipenem, meropenem, piperacillin-tazobactam, tobramycin
	Intrinsic*: Aztreonam, fosfomycin
EURGen-2023-02 (*E. coli*)	**Expected:** Amikacin, amoxicillin-clavulanic acid, ampicillin, aztreonam, cefepime, cefotaxime, ceftazidime, ceftazidime-avibactam, ciprofloxacin, ertapenem, fosfomycin, gentamicin, imipenem, meropenem, piperacillin-tazobactam, sulfamethoxazole, tobramycin, trimethoprim
	Expected non-mandatory: Colistin[a]
EURGen-2023-03 (*K. pneumoniae*)	**Expected:** Aztreonam, cefepime, cefotaxime, ceftazidime, ciprofloxacin
	Expected non-mandatory: Colistin[b]
	Intrinsic*: Ampicillin
EURGen-2023-04 (*P. aeruginosa*)	**Expected:** Amikacin, cefepime, ceftazidime, ceftazidime-avibactam, ciprofloxacin, gentamicin, imipenem, meropenem, piperacillin-tazobactam, tobramycin

[a] Detection of *pmrB* Y358N mutation, and subsequent inclusion of colistin in AMR profile of this strain, were expected results, but not mandatory to report
[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
[*] Intrinsic resistance (based on EUCAST Expected Phenotypes Version 1, February 2022), not part of the expected results

2.3. Distribution and procedure

On 31st March 2023, all laboratories that participate in the EURGen-RefLabCap project (n=39) were contacted by email and invited to participate in the 2023 EQA. The email contained a prenotification letter with a brief description of the exercise and indicated that deadline for signing up was 1st May 2023. In total, 31 laboratories signed up to participate in the 2023 EQA. On 6th June 2023, all EQA participants were sent an email confirming their registration and informing them that the exercise would start soon. On 15th June 2023, the test material (purified DNA) was shipped to the laboratories that signed up for the EQA 2023. On 15th June 2023, all EQA participants received an email with instructions on how to download the sequence data from the online platform ScienceData[19], 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[20]. In addition, the EQA participants received information on how to upload their sequencing data for the quality control evaluation. On 20th June 2023, participants received an email informing that the webtool for submission of results[21] was open, and that submission could take place until the deadline of 15th August 2023. 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 (15th August), 27 out of 31 laboratories had completed the EQA. The remaining laboratories were contacted individually to inquire on the status of their analyses and/or submission, and the deadline was extended by one week. The EQA was formally completed on 22nd August 2023, with results from 30 participating laboratories, representing 29 countries.

The webtool for submission of results has been developed and hosted by DTU for the purpose of similar EQAs and future related EQAs. The participants were asked to sequence the DNA using their desired sequencing platform and the routine methods implemented in their laboratory. They were asked to predict or detect: i) the ST; 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), only clinically relevant antimicrobials or those relevant for surveillance purposes should have been considered (Table 6).

Table 6. Relevant antimicrobials that should have been considered in the 2023 EQA, according to the bacterial species

Bacterial species	**Antimicrobials to consider**	**Nr.**
E. coli and *K. pneumoniae* (WS1 pathogens)	Amikacin, amoxicillin-clavulanic acid, ampicillin, aztreonam, cefepime, cefotaxime, ceftazidime, ceftazidime-avibactam, ciprofloxacin, colistin, ertapenem, fosfomycin, gentamicin, imipenem, meropenem, piperacillin-tazobactam, sulfamethoxazole, tigecycline, tobramycin, trimethoprim	20
A. baumannii and *P. aeruginosa* (WS2 pathogens)	Amikacin, aztreonam, cefepime, ceftazidime, ceftazidime-avibactam, ciprofloxacin, colistin, fosfomycin, gentamicin, imipenem, meropenem, piperacillin-tazobactam, tobramycin	13

[19] https://sciencedata.dk
[20] https://www.eurgen-reflabcap.eu/resources/eqa
[21] https://eurgen-reflabcap-pt.dtu.dk

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 ST 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"[22] and the "'Proposed common WGS-based genome analysis methods and standard protocols for national surveillance and integrated outbreak investigations of *Pseudomonas aeruginosa* and *Acinetobacter baumannii*" 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 four sets of results: one set of results for each strain, that could be obtained by sequencing the DNA samples and performing bioinformatics analysis or could be obtained by analysing the files produced at DTU either by short- or long-read sequencing (either FASTA or FASTQ files).

On 18th October 2023, 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. As another attachment, each participant that submitted locally generated sequencing data for quality control also received a report with the evaluation of the quality of their sequencing data. This email also contained a link to a feedback survey about the 2023 EQA, with a deadline of 6th November 2023.

2.4. Scoring system in the webtool

2.4.1. Overview of the scoring system for bioinformatics results

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, chromosomal PMs or antimicrobials that were not part of the expected results). Moreover, the webtool assigned a "blank" if the participants missed any genetic determinants, antimicrobials, or replicons that were part of the expected results. Scoring the missing expected determinant as "blank" is due to several situations which may not be considered as participant's mistake. As an example, an expected genetic determinant cannot be detected if the participants used a database in which that determinant is not included. Additionally, an expected determinant might also be missed due to stricter thresholds for identity and coverage are selected by the participants, compared to the thresholds used to prepare the expected results. Additionally, the webtool assigned a "blank" if the participants reported any AMR determinants, antimicrobials, or replicons that were not mandatory to be reported. These included AMR determinants for which there was no consensus between the bioinformatics tools while preparing the expected results, as well as antimicrobials for which the species is expected to be intrinsically resistant (intrinsic resistance) as specified in the EUCAST list of expected phenotypes[23] (Version 1.0, February 2022). The scoring system was the same as applied in the first EURGen-RefLabCap EQA in 2022, with the difference that the *in silico* prediction of AMR profiles was now evaluated individually for each antimicrobial, instead of being evaluated as a full profile. A complete description of the scoring system is provided in Table 7.

[22] www.eurgen-reflabcap.eu/resources/wgs-tools
[23] https://www.eucast.org/expert_rules_and_expected_phenotypes/expected_phenotypes

Table 7. Scoring system applied to the analyses included in the 2023 EQA

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

The maximum possible score that each laboratory could achieve 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 of 87 points. Table 8 shows the scores regarding each strain and type of analysis included in the 2023 EQA.

Table 8. Maximum possible score for the laboratories participating in the EQA, per strain and per type of analysis

Material and analysis	EURGen-2023-01	EURGen-2023-02	EURGen-2023-03	EURGen-2023-04	Total
Prediction of ST	1	1	1	1	4
Detection of plasmid replicons	0	6	1	0	7
Detection of AMR genes and chromosomal PMs	6	17	4	6	33
In silico prediction of AMR profiles	10	18	5	10	43
Total	**17**	**42**	**11**	**17**	**87**

2.4.2. 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 one situation where the reported unexpected genetic determinant had a very high genetic similarity to the expected gene. There was one situation where a score "0" deemed inappropriate was:

- Reporting *bla*$_{\text{OXA-31}}$ instead of *bla*$_{\text{OXA-4}}$ (due to a high genetic similarity (99.88% identity) between these two β-lactamase genes)

The scoring was manually adjusted in this situation to better reflect participant's ability to achieve the expected results defined for this EQA. The preliminary individual evaluation

report for each of the laboratories were already updated before the release and the participants no longer received a score "0" (which indicates an error), but instead were assigned a result of "1". 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.

2.5. Evaluation of sequences submitted by participants

In the EQA 2023, participants were also offered to submit the raw sequencing data that they generated using the DNA test material for quality evaluation. For submission of raw sequencing data (FASTQ files), participants were instructed to use the ScienceData platform[24]. Each participant was assigned a laboratory ID (Lab ID) and provided with a unique link to their individual folder on the ScienceData platform where they could upload their produced FASTQ files. Participants could submit sequences generated by short-read or long-read sequencing technologies and could submit either single-end or paired-end sequencing data.

All the sequencing data submitted by the participants was analyzed using standard bioinformatics tools. For the quality control (QC), genome assembly was performed using SPAdes v3.15.3[25] and the submitted sequences were compared with the reference genomes using sequence alignment program Burrows-Wheeler Aligner (BWA-MEM) v0.7.10[26]. The sequence mapping statistics were generated using samtools v1.2[27]. Summary assembly and read quality parameters were then produced with in-house pipelines. For long-read data, reads QC and filtering were done with Nanoq[28] v0.10.0, mapping to reference conducted with minimap2v2.24[29] and assembled with Flye[30] v2.9.1. For MLST, alleles and sequence types were predicted using the CGE MLST tool (MLST v2.0)[31].

A subset of quality parameters was used as indicator for general performance. These are widely used routine QC parameters and include: i) cgMLST, ii) MLST, iii) average coverage, iv) average Q-score, v) proportion of reads mapping to reference genome, vi) size of assembled genome compared to reference, vii) number of contigs above 200 bp, viii) genomic coverage with a minimum depth of 10x, ix) N50. An overview of the parameters used for scoring is presented in Table 9. A complete list of the quality parameters evaluated in the EQA 2023 are listed in Appendix 1 and 2.

[24] https://sciencedata.dk/
[25] https://github.com/ablab/spades
[26] https://github.com/lh3/bwa
[27] https://github.com/samtools/samtools
[28] https://github.com/esteinig/nanoq
[29] https://github.com/lh3/minimap2
[30] https://github.com/fenderglass/Flye
[31] https://cge.food.dtu.dk/services/MLST/

Table 9. Overview of the quality control parameters used for scoring and the maximum possible score for each parameter

Group	Metrics	Maximum score
Group 1	Q-score Forward reads (R1):	7.5
	Q-score Reverse Reads (R2):	7.5
	Depth of coverage: Chromosome:	5
	proportion of cgMLST match:	15
	MLST	10
Group 2	Size of assembled genome per total size of DNA sequence (%)	7.5
	Number of contigs > 200 bp	7.5
Group 3	Proportion of reads mapped to reference DNA sequence (%):	5
	Coverage 10x of the reference genome (%):	7.5
	N50	7.5
Bonus	Bonus group 2[a]	10
	Bonus group 3[b]	10
Total		**100**

[a] Pass all group 2 metrics
[b] Pass all group 3 metrics

3. RESULTS

3.1. Participating laboratories and analysed materials

All NRLs that participate in the EURGen-RefLabCap project (n=39) were invited to complete the EQA exercise. The number of laboratories that signed up for the EQA exercise was 31 and all laboratories submitted their results except for one (EURGen-RLC-005). Of 30 participants, two participants submitted results for only WS1 pathogens, and 28 laboratories submitted results for all pathogens. Most laboratories used DNA as test material (n=23), five laboratories analysed FASTQ files, and 2 laboratories analysed FASTA files corresponding to the DNA test material (Table 10). One laboratory used DNA for WS1 pathogens and FASTQ for WS2 pathogens. Overall, all laboratories submitted results for WS1 pathogens, while 28 laboratories submitted results for WS2 pathogens.

Table 10. Materials analysed in the EURGen-RefLabCap 2023 EQA, as reported by participating laboratories (n=30)**.**

Laboratory	**EURGen-2023-01 (*A. baumannii*)**	**EURGen-2023-02 (*E. coli*)**	**EURGen-2023-03 (*K. pneumoniae*)**	**EURGen-2023-04 (*P. aeruginosa*)**
EURGen-RLC-001	DNA	DNA	DNA	DNA
EURGen-RLC-002	DNA	DNA	DNA	DNA
EURGen-RLC-003	FASTQ files	FASTQ files	FASTQ files	FASTQ files
EURGen-RLC-004	DNA	DNA	DNA	DNA
EURGen-RLC-008	FASTA files	FASTA files	FASTA files	FASTA files
EURGen-RLC-009	DNA	DNA	DNA	DNA
EURGen-RLC-010	DNA	DNA	DNA	DNA
EURGen-RLC-011	DNA	DNA	DNA	DNA
EURGen-RLC-012	DNA	DNA	DNA	DNA
EURGen-RLC-014	DNA	DNA	DNA	DNA
EURGen-RLC-015	DNA	DNA	DNA	DNA
EURGen-RLC-016	DNA	DNA	DNA	DNA
EURGen-RLC-017	DNA	DNA	DNA	DNA
EURGen-RLC-018	FASTQ files	DNA	DNA	FASTQ files
EURGen-RLC-019	Not analyzed	DNA	DNA	Not analyzed
EURGen-RLC-020	DNA	DNA	DNA	DNA
EURGen-RLC-021	DNA	DNA	DNA	DNA
EURGen-RLC-022	DNA	DNA	DNA	DNA
EURGen-RLC-023	DNA	DNA	DNA	DNA
EURGen-RLC-024	FASTA files	FASTA files	FASTA files	FASTA files
EURGen-RLC-026	FASTQ files	FASTQ files	FASTQ files	FASTQ files
EURGen-RLC-027	Not analyzed	DNA	DNA	Not analyzed
EURGen-RLC-028	FASTQ files	FASTQ files	FASTQ files	FASTQ files
EURGen-RLC-029	DNA	DNA	DNA	DNA
EURGen-RLC-030	DNA	DNA	DNA	DNA
EURGen-RLC-031	DNA	DNA	DNA	DNA
EURGen-RLC-032	FASTQ files	FASTQ files	FASTQ files	FASTQ files
EURGen-RLC-033	DNA	DNA	DNA	DNA
EURGen-RLC-034	DNA	DNA	DNA	DNA
EURGen-RLC-035	FASTQ files	FASTQ files	FASTQ files	FASTQ files

3.2. Quality control of sequences submitted by participants

In total, 24 participants submitted their locally generated sequencing data for the QC. Most of the participants (n=22) submitted short-read sequencing data generated using Illumina (80 submissions (paired-end data)), while two laboratories submitted long-read sequencing data generated using Nanopore sequencing (8 submissions) (Table 11).

Table 11. Overview of sequencing technology used and the number of files submitted by each participating laboratory in the EURGen-RefLabCap 2023 EQA**.**

Laboratories	Sequencing platform	Single- or paired-end	Defined read-length (bp)	Files submitted for QC (n)
EURGen-RLC-001	iSeq (Illumina Inc.)	Paired-end	2 x 151	4
EURGen-RLC-002	MiniSeq (Illumina Inc.)	Paired-end	2 x 151	4
EURGen-RLC-004	MinION (Oxford Nanopore Technologies)	NA	NA	4
EURGen-RLC-009	NextSeq (Illumina Inc.)	Paired-end	2 X 151	4
EURGen-RLC-010	NextSeq (Illumina Inc.)	Paired-end	2 x 151	4
EURGen-RLC-011	MiSeq (Illumina Inc.)	Paired-end	2 x 251	4
EURGen-RLC-012	NextSeq (Illumina Inc.)	Paired-end	2 x 151	2
EURGen-RLC-014	MiSeq (Illumina Inc.)	Paired-end	2 x 151	4
EURGen-RLC-015	MiSeq (Illumina Inc.)	Paired-end	2 x 301	4
EURGen-RLC-016	NextSeq (Illumina Inc.)	Paired-end	2 x 151	4
EURGen-RLC-017	MiSeq (Illumina Inc.)	Paired-end	2 x 301	4
EURGen-RLC-018	MiSeq (Illumina Inc.)	Paired-end	NA	2
EURGen-RLC-019	NextSeq (Illumina Inc.)	Paired-end	2 x 151	2
EURGen-RLC-020	MiSeq (Illumina Inc.)	Paired-end	2 x 251	4
EURGen-RLC-021	MiniSeq (Illumina Inc.)	Paired-end	2 x 151	4
EURGen-RLC-022	MiSeq (Illumina Inc.)	Paired-end	NA	4
EURGen-RLC-023	MiSeq (Illumina Inc.)	Paired-end	2 x 251	4
EURGen-RLC-027	MiSeq (Illumina Inc.)	Paired-end	2 x 251	2
EURGen-RLC-029	NextSeq (Illumina Inc.)	Paired-end	2 x 301	4
EURGen-RLC-030	NextSeq (Illumina Inc.)	Paired-end	NA	4
EURGen-RLC-031	MiSeq (Illumina Inc.)	Paired-end	2 x 151	4
EURGen-RLC-032	MinION (Oxford Nanopore Technologies)	NA	NA	4
EURGen-RLC-033	MiSeq (Illumina Inc.)	Paired-end	2 x 151	4
EURGen-RLC-034	NextSeq (Illumina Inc.)	Paired-end	2 x 151	4

NA: Not applicable

The results of the evaluation of the quality parameters were shared with each participant by email along with the guide for self-evaluation and interpretation of results. In this report, an overview of Illumina quality parameters is presented for all the participants that submitted sequencing data for the QC analysis. The total number of submitted genomes included in the analysis was 80 (each submission consisting of short-read paired-end sequences), specifically 19 *A. baumannii*, 21 *E. coli*, 21 *K. pneumoniae* and 19 *P. aeruginosa* (Table 12).

Genomes underwent an initial screening and exclusion from the statistical analyses (Figure 2). The submissions which deviated more than 10% in assembly size compared to the reference genomes or had less than 95% of assigned cgMLST alleles were excluded from the statistical analyses (Figure 2). From initial screening, eight identified genomes were excluded from the statistical analyses when setting the QC thresholds (EURGen-2023-01 (n=1), EURGen-2023-02 (n=2), EURGen-2023-03 (n=2), EURGen-2023-04 (n=3)). While these submissions were excluded from the statistical analyses, but they were scored like all other submissions and the results were reported to the participants. Seven of these genomes belonged to two laboratories (Table 12).

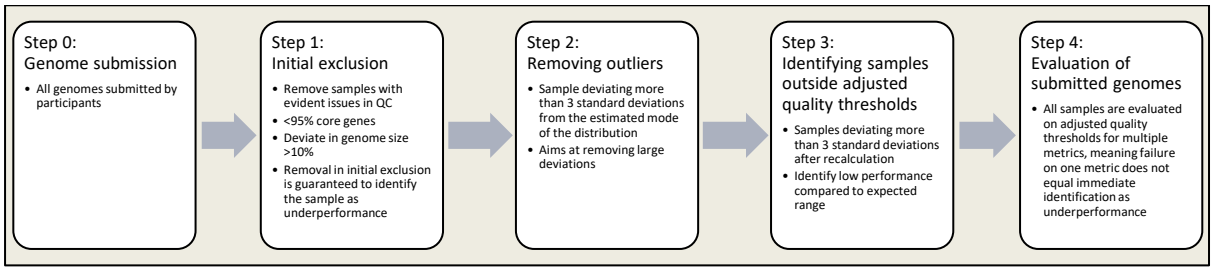

Figure 2. Overview of steps in the data analysis and calculation of thresholds for WGS data evaluation

Overall, most laboratories performed satisfactorily. Of 22 laboratories that submitted the Illumina sequences for QC analysis, most of the laboratories (n=17) achieved scores of 95% or higher of their maximum possible score. Overall, the participants achieved scores which correspond to 35% to 100% of their maximum possible score. For EURGen-2023-02 and EURGen-2023-03, 17 and 18 laboratories achieved a score of 95% or higher of their maximum possible score, respectively. For EURGen-2023-01 and EURGen-2023-04, 16 laboratories achieved a score of 95% or higher of their maximum possible score. One laboratory achieved a score below 60% of their maximum possible score for all four test strains. Six laboratories achieved 100% of their maximum possible scores for all the submitted sequences (Figure 3, Table 12).

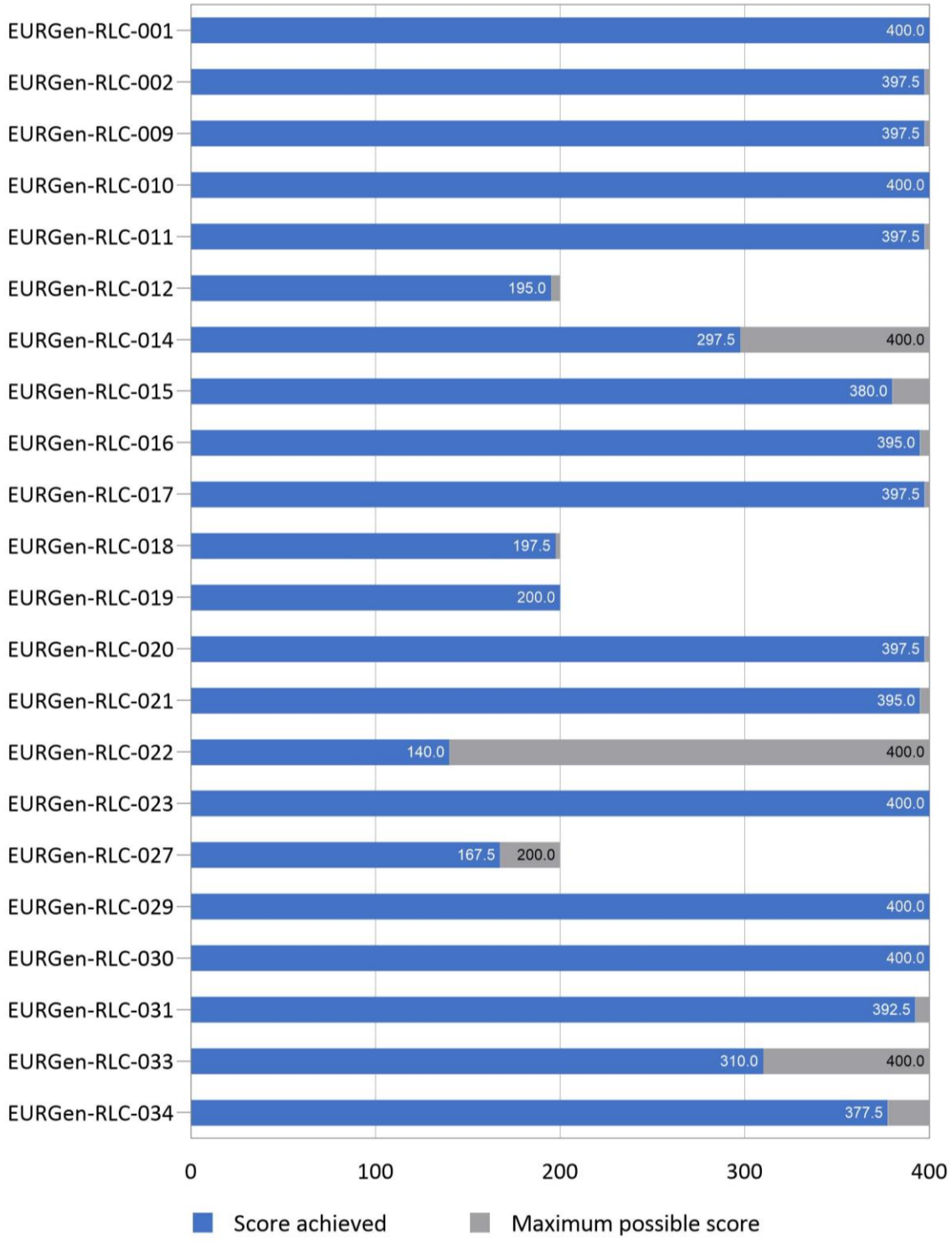

Figure 3. Summary of maximum possible scores and total achieved scores from the QC analyses of Illumina short-read WGS data submitted by the participants in the EQA 2023. The maximum possible score for each laboratory varies depending on the number of sequencing files submitted by the participants and eventually included in the analysis after passing the initial screening.

Table 12. Maximum possible scores and scores obtained by each participant, for each Illumina sequence file submitted, and in total

Laboratories	EURGen-2023-01	EURGen-2023-02	EURGen-2023-03	EURGen-2023-04	Max. possible score	Obtained Score	Score (%)
EURGen-RLC-001	100	100	100	100	400	400	100
EURGen-RLC-002	100	100	97.5	100	400	397.5	99.4
EURGen-RLC-009	100	100	100	97.5	400	397.5	99.4
EURGen-RLC-010	100	100	100	100	400	400	100
EURGen-RLC-011	100	100	100	97.5	400	397.5	99.4
EURGen-RLC-012	97.5	NA	NA	97.5	200	195	97.5
EURGen-RLC-014	75*	75*	75*	72.5*	400	297.5	74.4
EURGen-RLC-015	100	80	100	100	400	380	95
EURGen-RLC-016	100	100	100	95	400	395	98.8
EURGen-RLC-017	100	100	100	97.5	400	397.5	99.4
EURGen-RLC-018	NA	100	97.5	NA	200	197.5	98.8
EURGen-RLC-019	NA	100	100	NA	200	200	100
EURGen-RLC-020	100	100	100	97.5	400	397.5	99.4
EURGen-RLC-021	100	100	97.5	97.5	400	395	98.8
EURGen-RLC-022	55	25*	25*	35*	400	140	35
EURGen-RLC-023	100	100	100	100	400	400	100
EURGen-RLC-027	NA	95	72.5	NA	200	167.5	83.8
EURGen-RLC-029	100	100	100	100	400	400	100
EURGen-RLC-030	100	100	100	100	400	400	100
EURGen-RLC-031	100	97.5	97.5	97.5	400	392.5	98.1
EURGen-RLC-033	75	100	100	35*	400	310	77.5
EURGen-RLC-034	100	77.5	100	100	400	377.5	94.4
Averages	**94.9**	**92.9**	**93.5**	**90.5**	**NA**	**NA**	**93.1**

NA; Not applicable

* One or more submissions from these laboratories were excluded from the statistical analyses after the initial screening.

Overall, submitted genomes were within the minimum cut-off values for almost all the submissions (n=65). Seven submitted genomes were outside adjusted quality thresholds, four of which were also identified as outliers (Figure 4 and 5). Outliers were defined as data points that were more than three standard deviations from the estimated mode of the distribution, after the initial exclusions of genomes. After removing the outliers, thresholds were recalculated using the same methodology, this is referred to as adjusted quality thresholds. For metrics without a predefined cut-off, the adjusted quality thresholds were utilized for identifying laboratories not performing comparably to the general standard quality seen among participants. The adjusted quality thresholds were used for the metrics; size of assembled genome compared to reference, number of contigs above 200bp, genomic coverage with a minimum depth of 10x, and N50.

The average phred scores (Q-score) of the submitted raw reads were evaluated. For the forward reads, the average Q-scores for all the sequences for all the test strains were above the preferred cut-off value i.e., >Q30. For reverse reads, only the sequences from *A. baumannii* (EURGen-2023-01) had all submission above preferred cut-off for the average Q-score. Seven reverse reads files for test strains EURGen-2023-02 (n=2), EURGen-2023-03 (n=3) and EURGen-2023-04 (n=2) had average Q-scores below the preferred threshold. For all the test strains, all sequences (both forward (R1) and reverse reads (R2)) had average Q-scores above the minimum cut-off value i.e., >Q25, thus no sequence data from all the participants were identified as unsatisfactory (Figure 4A and 4B).

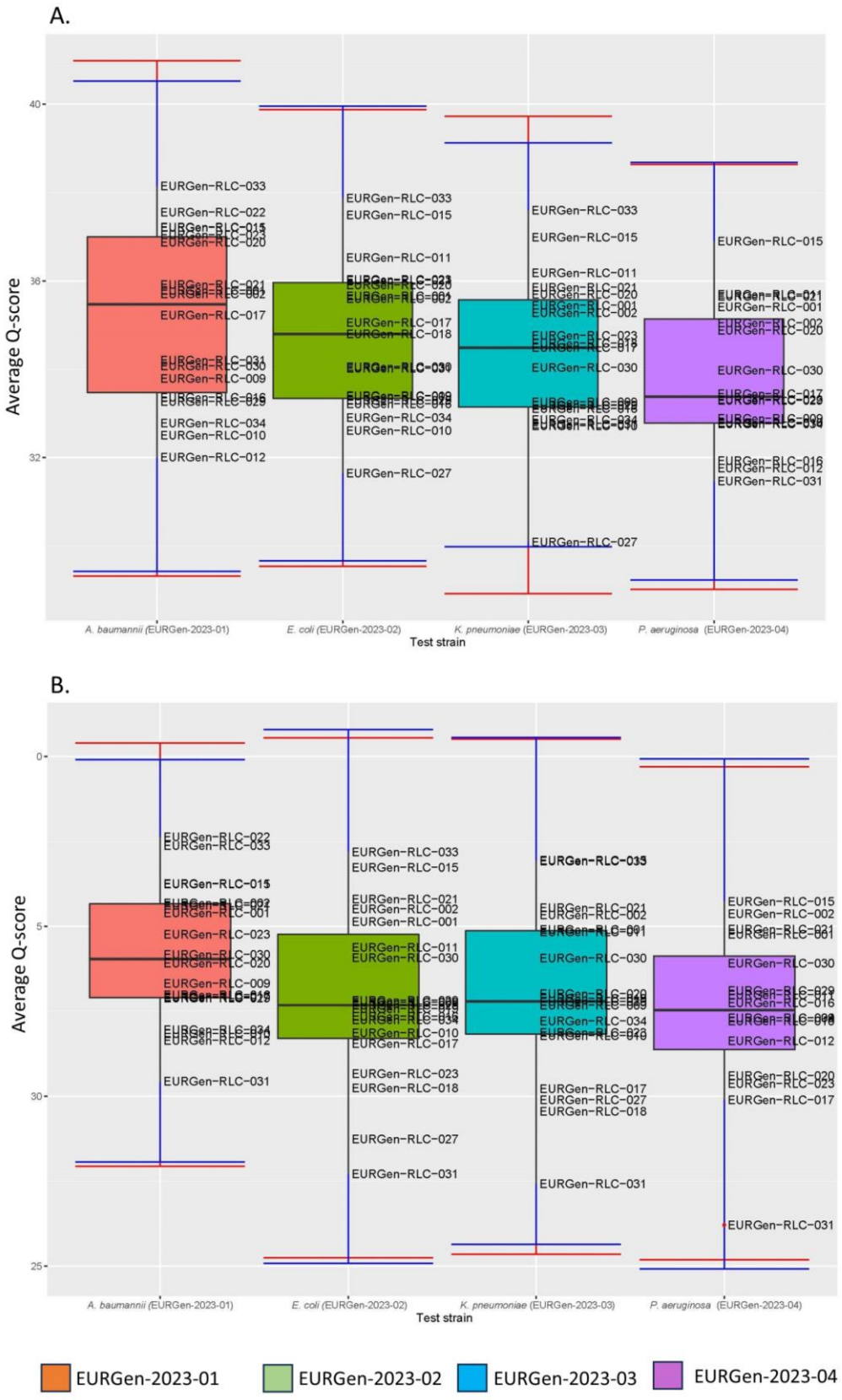

Figure 4. Box plots of average phred score (Q-score) of the raw reads (short read sequencing) submitted by the participants for the evaluation (n=21). Red whiskers show 3 standard deviations from the mean. Blue whiskers show the recalculated 3 standard deviations (referred to as adjusted quality threshold). The dataset used for calculating the adjusted quality threshold removes samples which were identified as outliers in any metric. A) Boxplot of average Q-score for forward reads (R1). B) Boxplot of average Q-score for reverse reads (R2).

Contigs less than 200 bp are expected to be non-informative and likely due to the artefacts or residual contaminants from the library preparation step. These contigs are routinely excluded from analysis. The number of contigs above 200 bp should be as low as possible in an assembly, and large number of short contigs indicate a problem with the raw sequence data leading to poor assembly. For the analysis, the adjusted quality threshold value of contigs above 200 bp was set at three standard deviations above the median. For EURGen-2023-02, EURGen-2023-03 and EURGen-2023-04, all assemblies performed satisfactorily, and the number of contigs above 200 bp were below the cut-off values. For EURGen-2023-01, the number of contigs above 200 bp in one submitted genome were higher than the cut-off values (Figure 5A).

For the genomic coverage of the 10X of the reference genomes, minimum cut-off value was set at three standard deviations below the median. A coverage depth of 10X at one base position means that 10 sequenced reads cover that base, while the genomic coverage of 10X of the genome represents the percentage of the entire genomic DNA which has at least 10X coverage depth. Overall, most laboratories (n=16) have a genomic coverage above the adjusted quality threshold. For EURGen-2023-01 (n=2), EURGen-2023-02 (n=2) and EURGen-2023-03 (n=1), genomes were found outside accepted cut-offs. Each was from a different laboratory and for each species one was also identified as an outlier. For EURGen-2023-04, the datasets from all the laboratories have a genomic coverage above 99% and were inside expected range. The datasets from most laboratories have a genomic coverage of above 99% for EURGen-2023-02 (n=17) and EURGen-2023-03 (n=18) (Figure 5B).

The proportion of reads that align directly to the closed reference genomes was also evaluated for 21 laboratories that submitted the short-read sequencing data. This metric indicates the amount of possible contamination and non-sense reads in the datasets. The minimum cut-off value was defined as >80%, and preferred cut-off value was set at 90%. Overall, all the laboratories had high proportion (more than 80%) of reads that mapped to the reference genome. For EURGen-2023-01, EURGen-2023-02 and EURGen-2023-03, most laboratories (n=20) had more than 90% of reads that mapped to the reference genome. For EURGen-2023-04, 15 laboratories had more than 90% of reads that mappped to the reference genome and a wider distribution (Figure 5C).

For comparing the size of assembled genomes with the reference genomes, proportion of assembly size compared to the reference genome was evaluated for 22 laboratories. The assembly size should be close to 100%, but can deviate due to genomic complexity, such as repeated sequences. The minimum cut-off values were defined as 3 standard deviations from the mean. For EURGen-2023-01, EURGen-2023-02 and EURGen-2023-03, all laboratories (n=21) performed satisfactorily, and the size of assemblies was within the adjusted quality thresholds. For EURGen-2023-04, the size of two assemblies was above the cut-off value and one was an outlier. Notably, these were only marginally larger than the expected size, but compared to all submitted results were 0.5-1.5% larger than majority of submissions (Figure 5D).

The most common parameter with insufficient quality was coverage 10x. In summary, laboratories remarked for genomes submitted with QC insubstantialities: EURGen-RLC-14, due to number of contigs and assembly sizes across all four submitted genomes. EURGen-RLC-15, due to coverage 10x of sample EURGen-2023-02. EURGen-RLC-22 due to multiple critical QC parameters across all four isolates. EURGen-RLC-27 due to lack of coverage and depth of sample EURGen-2023-03. EURGen-RLC-33 due to coverage 10x and N50 of samples EURGen-2023-01 and coverage 10x, depth, contigs and N50 of EURGen-2023-04. EURGen-RLC-34 due to coverage 10x and depth of EURGen-2023-02.

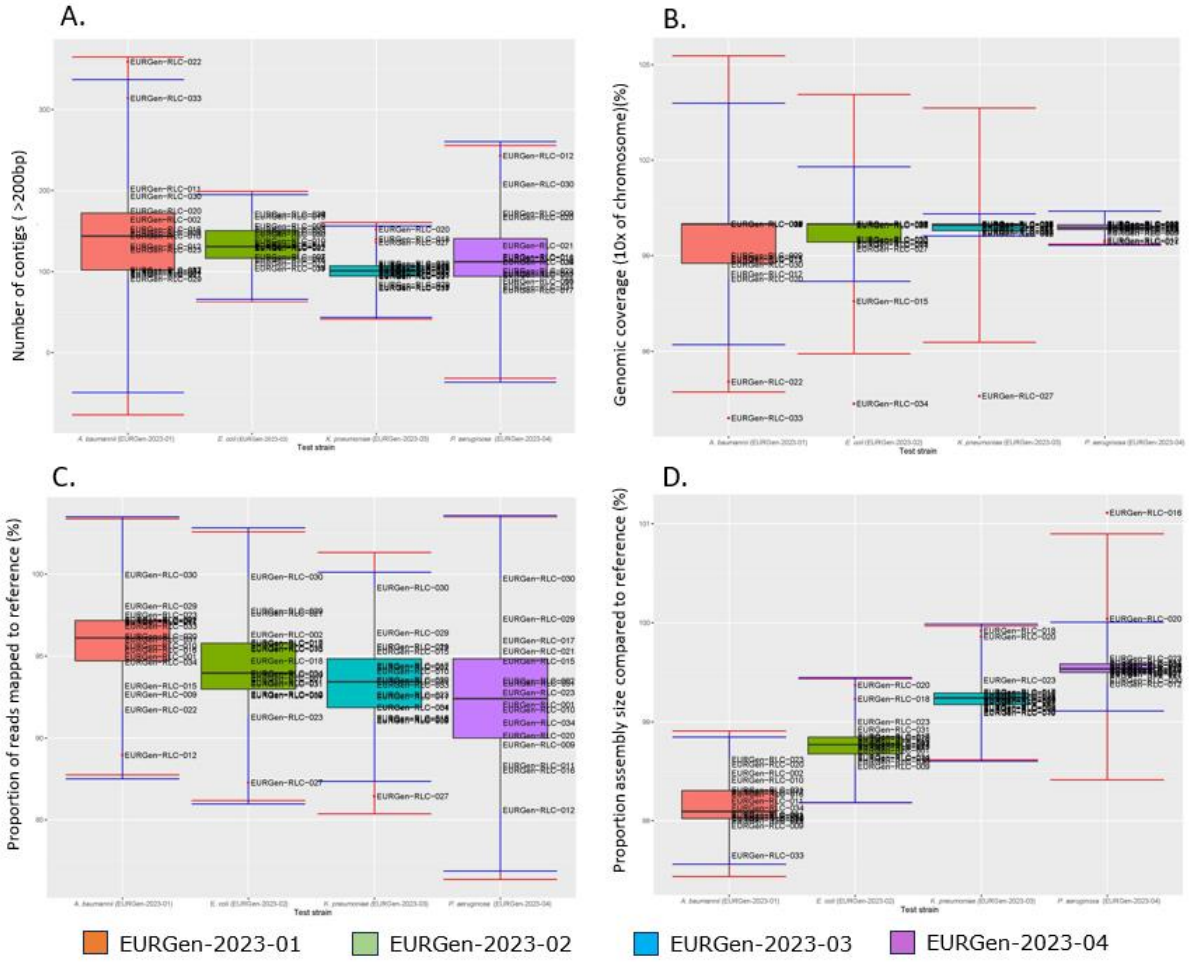

Figure 5. Box plots of some of the quality control metrics for the evaluation of Illumina sequences submitted by the participants (n=21). Red whiskers show the thresholds for identification of outliers. Blue whiskers show the thresholds for identification of underperformance (referred to as adjusted quality threshold). The dataset used for calculating the adjusted quality threshold removes samples which were identified as outliers in any metric. A) Boxplot of total number of contigs of the assembled genomes for each test strain. B) Boxplot of genomic coverage represented as 10x of the chromosome (%). C) Boxplot of the proportion of reads mapped directly to the closed reference genome (%). D) Boxplot of the size of assembly compared to the size of the reference genome.

Long-read sequences (n=2) from two laboratories were evaluated likewise. The QC definitions for Oxford Nanopore Technologies sequences are poorly defined as the technology is under continuous development, and the low number of submissions does not allow for statistical inter-laboratory comparison. Based on previous experiences with Nanopore technology and expert recommendations, submissions were evaluated on an individual basis. Overall, the main problem with the long-read sequences was lack of sequencing yield and the sequence samples were below the recommended coverage of 30X.

3.3. Overall scores and evaluation of submitted results

All participating laboratories correctly identified the species of the four strains included in the 2023 EQA. Most participants (n=28) submitted results for all four types of analysis included in this EQA, except for two participants that did not submit results for *in silico* prediction of AMR profiles, for any strain (Table 13). For all analyses evaluated in this EQA, the concordance between submitted and expected results varied between 58.6% and 100% (Figure 6, Table 13). 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 13). The descriptions of analysis-specific results are provided in the following sections.

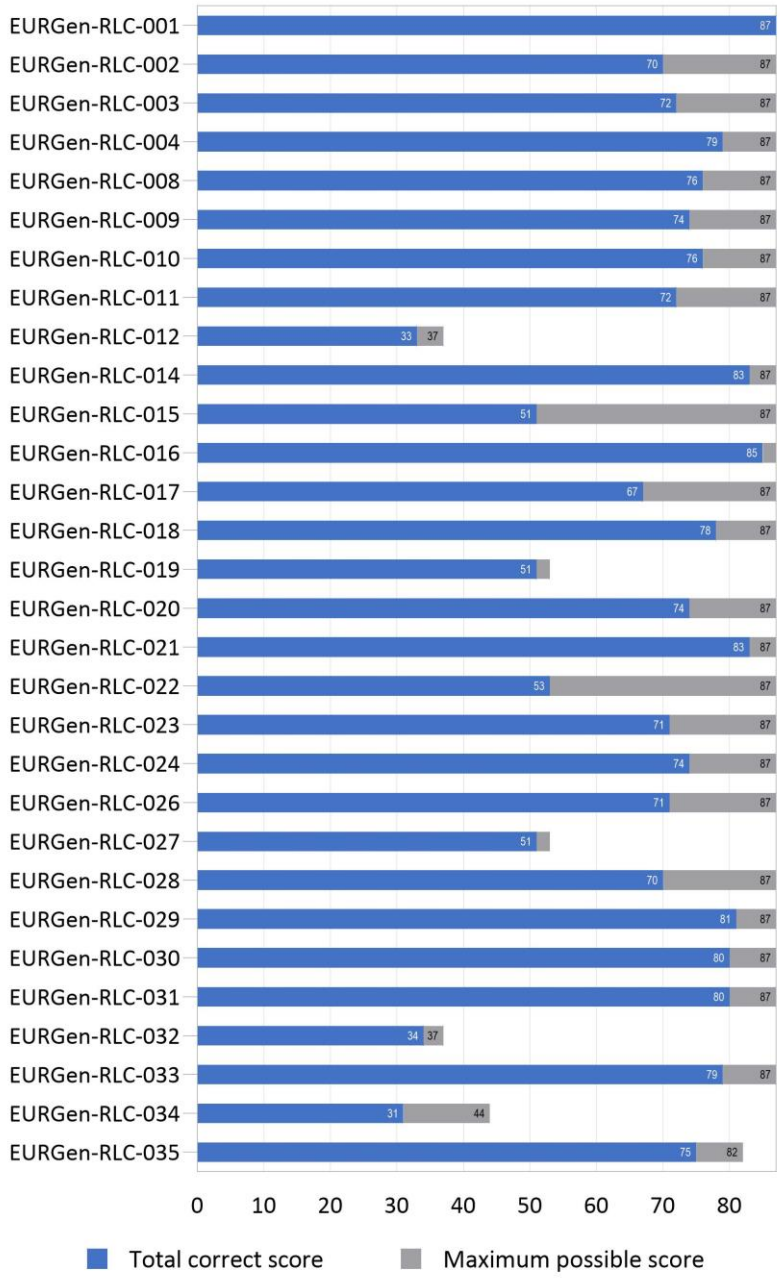

Figure 6. Concordance between submitted and expected results for all the analysis included in EQA 2023. The maximum possible score for each laboratory varies depending on the number of analyses that they performed and for how many strains they performed those analyses.

Table 13. Maximum possible scores and scores obtained by each participant, for each type of bioinformatics analysis included in the 2023 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	Obtained Score	Score (%)	Maximum possible score	Obtained Score	Score (%)	Maximum possible score	Obtained Score	Score (%)	Maximum possible score	Obtained Score	Score (%)	Maximum possible score	Obtained Score	Score (%)
EURGen-RLC-001	4	4	100	7	7	100	33	33	100	43	43	100	87	87	100
EURGen-RLC-002	4	4	100	7	7	100	33	22	66.7	43	38	88.4	87	71	81.6
EURGen-RLC-003	4	3	75	7	7	100	33	25	75.8	43	37	86	87	72	82.8
EURGen-RLC-004	4	4	100	7	5	71.4	33	28	84.8	43	42	97.7	87	79	90.8
EURGen-RLC-008	4	4	100	7	7	100	33	28	84.8	43	38	88.4	87	77	88.5
EURGen-RLC-009	4	3	75	7	6	85.7	33	28	84.8	43	37	86	87	74	85.1
EURGen-RLC-010	4	3	75	7	6	85.7	33	31	93.9	43	37	86	87	77	88.5
EURGen-RLC-011	4	3	75	7	7	100	33	25	75.8	43	37	86	87	72	82.8
EURGen-RLC-012	2	2	100	NA	NA	NA	12	11	91.7	23	20	87	37	33	89.2
EURGen-RLC-014	4	4	100	7	7	100	33	32	97	43	41	95.3	87	84	96.6
EURGen-RLC-015	4	3	75	7	4	57.1	33	28	84.8	43	16	37.2	87	51	58.6
EURGen-RLC-016	4	4	100	7	7	100	33	31	93.9	43	43	100	87	85	97.7
EURGen-RLC-017	4	3	75	7	5	71.4	33	23	69.7	43	36	83.7	87	67	77
EURGen-RLC-018	4	4	100	7	7	100	33	27	81.8	43	41	95.3	87	79	90.8
EURGen-RLC-019	2	2	100	7	7	100	21	21	100	23	21	91.3	53	51	96.2
EURGen-RLC-020	4	3	75	7	5	71.4	33	30	90.9	43	33	76.7	87	71	81.6
EURGen-RLC-021	4	3	75	7	7	100	33	32	97	43	41	95.3	87	83	95.4
EURGen-RLC-022	4	4	100	7	1	14.3	33	21	63.6	43	30	69.8	87	56	64.4
EURGen-RLC-023	4	4	100	7	6	85.7	33	30	90.9	43	35	81.4	87	75	86.2
EURGen-RLC-024	4	3	75	7	7	100	33	24	72.7	43	41	95.3	87	75	86.2

Analysis	Prediction of MLST			Detection of plasmid replicons			Detection of genetic AMR determinants			Prediction of AMR profiles			Totals		
Participants	Maximum possible score	Obtained Score	Score (%)	Maximum possible score	Obtained Score	Score (%)	Maximum possible score	Obtained Score	Score (%)	Maximum possible score	Obtained Score	Score (%)	Maximum possible score	Obtained Score	Score (%)
EURGen-RLC-026	4	3	75	7	4	57.1	33	27	81.8	43	38	88.4	87	72	82.8
EURGen-RLC-027	2	2	100	7	7	100	21	20	95.2	23	22	95.7	53	51	96.2
EURGen-RLC-028	4	3	75	7	7	100	33	25	75.8	43	35	81.4	87	70	80.5
EURGen-RLC-029	4	4	100	7	7	100	33	32	97	43	39	90.7	87	82	94.3
EURGen-RLC-030	4	4	100	7	6	85.7	33	30	90.9	43	41	95.3	87	81	93.1
EURGen-RLC-031	4	4	100	7	7	100	33	29	87.9	43	41	95.3	87	81	93.1
EURGen-RLC-032	4	4	100	NA	NA	NA	33	30	90.9	NA	NA	NA	37	34	91.9
EURGen-RLC-033	4	3	75	7	7	100	33	33	100	43	37	86	87	80	92
EURGen-RLC-034	4	4	100	7	5	71.4	33	22	66.7	NA	NA	NA	44	31	70.5
EURGen-RLC-035	4	4	100	7	7	100	33	31	93.9	38	32	84.2	82	74	90.2
Averages	**NA**	**3**	**90**	**NA**	**6**	**87.8**	**NA**	**27**	**86**	**NA**	**33.1**	**87.3**	**NA**	**69**	**86.8**

NA: Not applicable

3.4. Prediction of sequence types

Participants used both publicly available and commercial software and/or databases for prediction of the ST. The most reported software was CGE MLST and its respective database. A full description of the methods reported by the participants is provided in Appendix 3.

In total, out of 120 possible MLST result submissions, 113 were submitted by all participating laboratories. Of 30 participants, 27 submitted results for all four test strains. Two participants (EURGen-RLC-019 and EURGen-RLC-027) did not submit MLST results for WS2 pathogens, while one participant (EURGen-RLC-012) did not submit results for WS1 pathogens. In addition, one participant (EURGen-RLC-021) did not submit results for the test strain EURGen-2023-01.

A total of 113 MLST predictions were submitted, and these included the ST predictions for strains EURGen-2023-01 (n=27), EURGen-2023-02 (n=29), EURGen-2023-03 (n=29) and EURGen-2023-04 (n=28) (Table 14, Figure 7). Of the submitted 113 MLST predictions, 102 were correct (90.3%).

All ST predictions submitted for the strains EURGen-2023-02, EURGen-2023-03 and EURGen-2023-04 were correct. In total, 11 incorrect results (9.7%) were submitted for ST predictions, and all these incorrect results were submitted for the *A. baumannii* strain EURGen-2023-01.

Overall, 19 participants correctly identified the ST of all strains. Participants obtained between 75 % to 100 % of their maximum possible scores. The average concordance between expected and submitted results was 90% (Table 13, Figure 6).

Table 14. Distribution of submitted results regarding the prediction of ST

Test strains	Correct ST	Incorrect ST	Empty ST	Total
EURGen-2023-01	16	11	3	**30**
EURGen-2023-02	29	0	1	**30**
EURGen-2023-03	29	0	1	**30**
EURGen-2023-04	28	0	2	**30**
Total	**102**	**11**	**7**	**120**

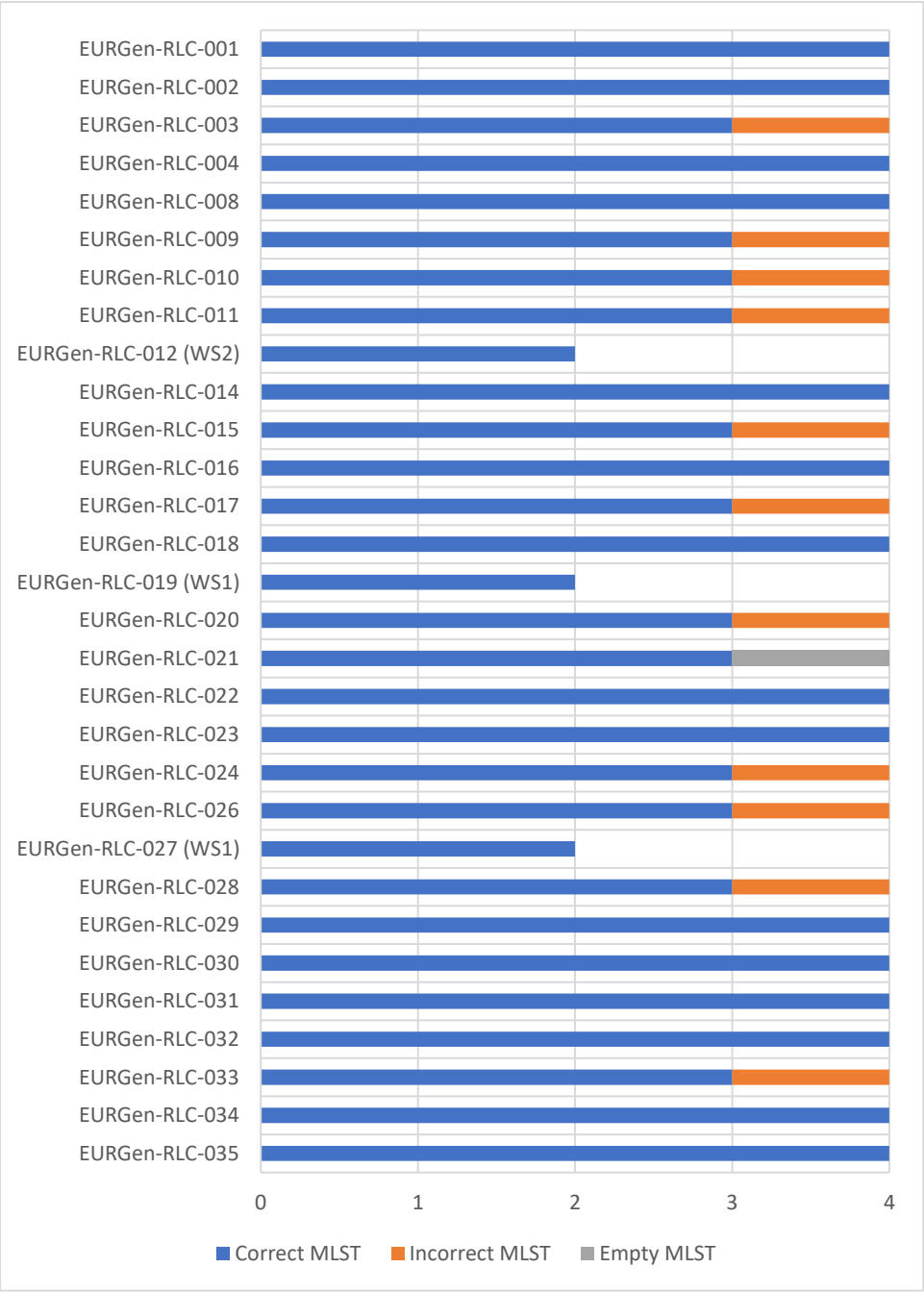

Figure 7. Distribution of submitted results regarding the prediction of ST. WS1: Only submitted results for workstream 1 pathogens; WS2: Only submitted results for workstream 2 pathogens.

3.5. Detection of plasmid replicon types

Participants used both publicly available and commercial software and/or databases for detection of the plasmid replicons. The most reported software was CGE PlasmidFinder and its respective database. A full description of the methods reported by the participants is provided in Appendix 4.

In total, 56 sets (28 laboratories) of results were submitted regarding the detection of plasmid replicon types. The submitted results were distributed equally between the two strains EURGen-2023-02 and EURGen-2023-03 (n=28 sets of results per strain). For

EURGen-2023-01 and EURGen-2023-04, no plasmid replicons were expected, and participants did not report any replicon gene for these strains.

Of the 56 sets of results submitted for the detection of plasmid replicons, 53.7% were fully correct (n=30). Additionally, in 28.6% of the sets of results (n=16), certain expected plasmid replicons were missing, and in 25% of the submitted results (n=14), 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 (7.1% or n=4) (Table 15, Figure 8).

Overall, 10 participants correctly identified all expected replicons for both strains, and no unexpected replicon was reported. The participants obtained between 1 and 7 points for the detection of plasmid replicons, which corresponded to 14.3% to 100 % of their maximum possible scores (7 points for each participant). The average concordance between expected and submitted results was 87.8% (Table 13, Figure 6).

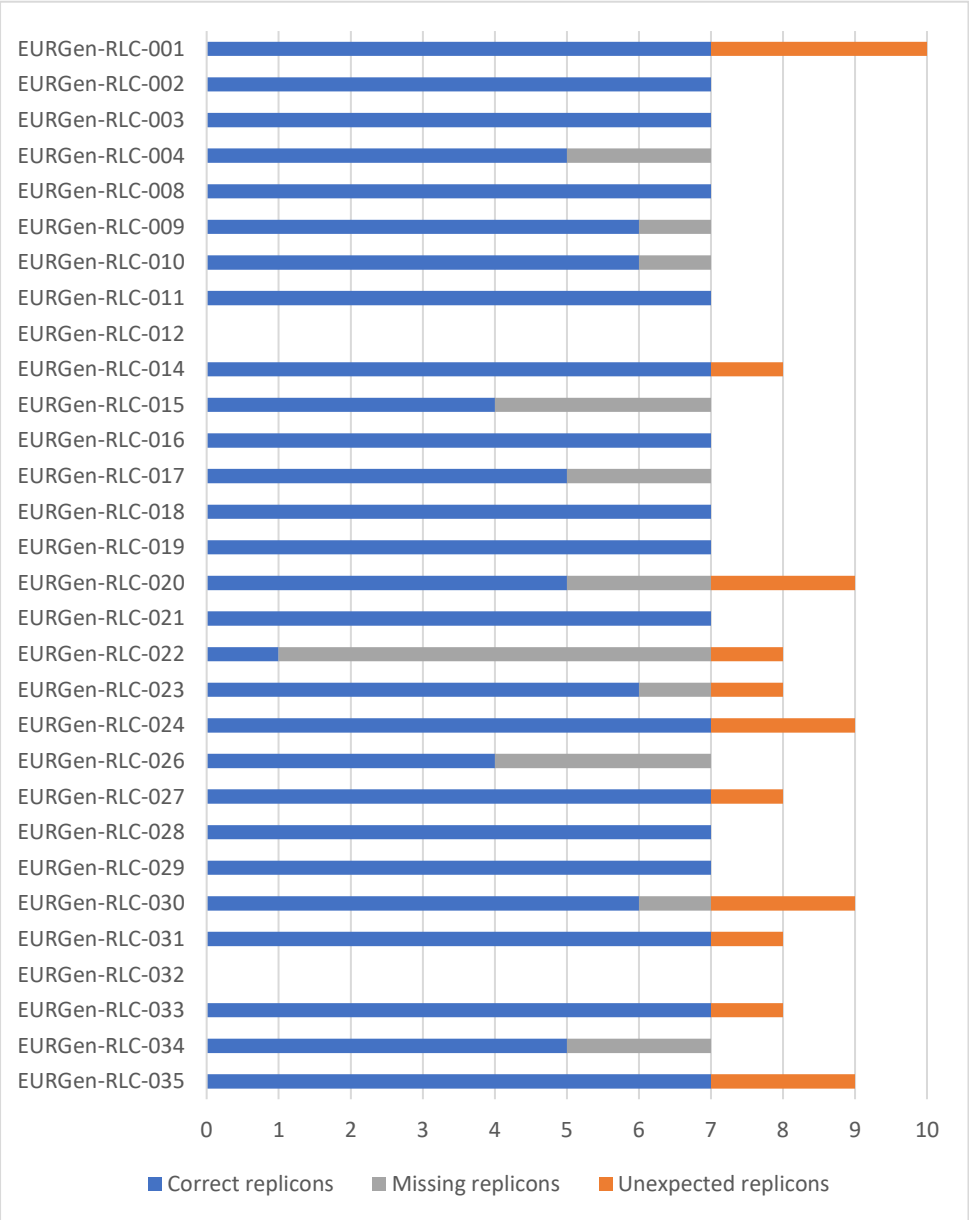

Figure 8. Distribution of submitted results regarding the detection of plasmid replicons.

Table 15. Distribution of submitted sets of results regarding the detection of plasmid replicons

Test strain	Correct replicons	Only missing replicons	Only unexpected replicons	Missing+un-expected replicons	Total
EURGen-2023-01	NA	NA	NA	NA	**NA**
EURGen-2023-02	13	7	6	2	**28**
EURGen-2023-03	16	4	6	2	**28**
EURGen-2023-04	NA	NA	NA	NA	**NA**
Total	**29**	**11**	**12**	**4**	**56**

NA; Not applicable

For strain EURGen-2023-02, participants were expected to detect six plasmid replicons (Col(BS512), ColKP3, IncFIA, IncFIB(AP001918), IncFII(pAMA1167-NDM-5), IncX3), while two expected replicons (Col(pHAD28 and IncQ1) were non-mandatory to report and yielded a "blank" score when reported. The replicon ColKP3 was not reported by six participants, while five participants did not report replicon IncFIB(AP001918). There were seven more cases of other missing replicons from the submitted results. Overall, 13 participants (50%) correctly reported all expected plasmid replicons, while two participants reported all expected replicons except ColKP3. The total number of missing replicons (excluding non-mandatory replicons) was 18. Additionally, for the non-mandatory expected replicons, six participants reported replicon Col(pHAD28), and 13 participants reported replicon IncQ1. Furthermore, six participants reported three additional unexpected replicons, i.e., ColpVC (n=3), IncFII(pRSB107) (n=2) and IncFIA(HI1) (n=1). The total number of unexpected replicons throughout all sets of submitted results was six. A complete description of the concordances and discordances between the expected plasmid replicons and the results submitted by participants is provided in Table 16.

Table 16. Results of the detection of plasmid replicons for each participant, for strain EURGen-2023-02 (*E. coli*)

	Expected								Unexpected						
Laboratories	Col(BS512)	Col(pHAD28)[a]	ColKP3	IncFIA	IncFIB(AP001918)	IncFII(pAMA1167-NDM-5)	IncQ1[a]	IncX3	ColpVC	IncFII(pRSB107)	IncFIA(HI1)	**Correct (nr.)**	**Missing (nr.)**	**Unexpected (nr.)**	**Expected non-mandatory (nr.)**
EURGen-RLC-001	x	-	x	x	x	x	x	x	x			6	0	1	1
EURGen-RLC-002	x	-	x	x	x	x	-	x				6	0	0	0
EURGen-RLC-003	x	-	x	x	x	x	-	x				6	0	0	0
EURGen-RLC-004	-	-	x	x	-	x	x	x				4	2	0	1
EURGen-RLC-008	x	-	x	x	x	x	x	x				6	0	0	1
EURGen-RLC-009	x	x	-	x	x	x	-	x				5	1	0	1
EURGen-RLC-010	x	-	x	x	-	x	x	x				5	1	0	1
EURGen-RLC-011	x	-	x	x	x	x	-	x				6	0	0	0
EURGen-RLC-014	x	-	x	x	x	x	x	x		x		6	0	1	1
EURGen-RLC-015	x	-	-	x	-	x	-	x				4	2	0	0
EURGen-RLC-016	x	-	x	x	x	x	x	x				6	0	0	1
EURGen-RLC-017	x	-	x	x	x	-	-	x				5	1	0	0
EURGen-RLC-018	x	-	x	x	x	x	-	x				6	0	0	0
EURGen-RLC-019	x	x	x	x	x	x	x	x				6	0	0	2
EURGen-RLC-020	x	-	-	x	x	x	x	x	x			5	1	1	1
EURGen-RLC-021	x	-	x	x	x	x	-	x				6	0	0	0
EURGen-RLC-022	x	-	-	-	-	-	-	-			x	1	5	1	0
EURGen-RLC-023	x	x	-	x	x	x	-	x				5	1	0	1
EURGen-RLC-024	x	-	x	x	x	x	x	x				6	0	0	1
EURGen-RLC-026	x	-	-	-	-	x	-	x				3	3	0	0
EURGen-RLC-027	x	x	x	x	x	x	-	x				6	0	0	1
EURGen-RLC-028	x	-	x	x	x	x	-	x				6	0	0	0
EURGen-RLC-029	x	-	x	x	x	x	x	x				6	0	0	1
EURGen-RLC-030	x	x	x	x	x	x	x	x	x			6	0	1	2
EURGen-RLC-031	x	-	x	x	x	x	x	x		x		6	0	1	1
EURGen-RLC-033	x	x	x	x	x	x	-	x				6	0	0	1
EURGen-RLC-034	x	-	x	x	x	-	-	x				5	1	0	0
EURGen-RLC-035	x	-	x	x	x	x	x	x				6	0	0	1
EURGen-RLC-012												0	0	0	0
EURGen-RLC-032												0	0	0	0
Correct (nr.)	27	6	22	26	23	25	13	27	NA	NA	NA		Total		
Missing or UN (nr.)	1	22	6	2	5	3	15	1	3	2	1	150	18	6	19

Cells shaded in green (x): Plasmid replicon reported
Cells shaded in red (-): Plasmid replicon missing
Cells shaded in orange (x): Unexpected plasmid replicon reported
Cells shaded in grey (/): participant did not submit plasmid replicons
NA: Not applicable; UN: Unexpected
[a] Expected but non-mandatory to report

For strain EURGen-2023-03, participants were expected to detect one plasmid replicon (repB(R1701)), while one replicon (Col(pHAD28)) was not mandatory to report and yielded a "blank" score when reported. Of 28 participants, the expected replicon repB(R1701) was not reported by six participants (21.4%). The non-mandatory replicon Col(pHAD28) was

reported by 11 participants (39.3%). Furthermore, two unexpected plasmid replicons (Col(MG828) and Col440I) were reported by eight participants. The most reported unexpected replicon was Col(MG828) (n=8), followed by Col440I (n=3). The total number of unexpected replicons throughout all sets of submitted results was 11 (Table 17).

Table 17. Results of the detection of plasmid replicons for each participant, for strain EURGen-2023-03 (*K. pneumoniae*)

	Expected		Unexpected					
Laboratories	Col(pHAD28)[a]	repB(R1701)	Col(MG828)	Col440I	**Correct (nr.)**	**Missing (nr.)**	**Unexpected (nr.)**	**Expected non-mandatory (nr.)**
EURGen-RLC-001	x	x	x	x	1	0	2	1
EURGen-RLC-002	-	x			1	0	0	0
EURGen-RLC-003	-	x			1	0	0	0
EURGen-RLC-004	x	x			1	0	0	1
EURGen-RLC-008	-	x			1	0	0	0
EURGen-RLC-009	x	x			1	0	0	1
EURGen-RLC-010	-	x			1	0	0	0
EURGen-RLC-011	-	x			1	0	0	0
EURGen-RLC-014	x	x			1	0	0	1
EURGen-RLC-015	-	-			0	1	0	0
EURGen-RLC-016	x	x			1	0	0	1
EURGen-RLC-017	-	-			0	1	0	0
EURGen-RLC-018	-	x			1	0	0	0
EURGen-RLC-019	x	x			1	0	0	1
EURGen-RLC-020	-	-	x		0	1	1	0
EURGen-RLC-021	-	x			1	0	0	0
EURGen-RLC-022	-	-			0	1	0	0
EURGen-RLC-023	x	x	x		1	0	1	1
EURGen-RLC-024	-	x	x	x	1	0	2	0
EURGen-RLC-026	-	x			1	0	0	0
EURGen-RLC-027	x	x	x		1	0	1	1
EURGen-RLC-028	-	x			1	0	0	0
EURGen-RLC-029	-	x			1	0	0	0
EURGen-RLC-030	x	-	x		0	1	1	1
EURGen-RLC-031	x	x			1	0	0	1
EURGen-RLC-033	x	x	x		1	0	1	1
EURGen-RLC-034	-	-			0	1	0	0
EURGen-RLC-035	-	x	x	x	1	0	2	0
EURGen-RLC-012					0	0	0	0
EURGen-RLC-032					0	0	0	0
Correct (nr.)	11	22	NA	NA		Total		
Missing or UN (nr.)	17	6	8	3	22	6	11	11

Cells shaded in green (x): Plasmid replicon reported
Cells shaded in red (-): Plasmid replicon missing
Cells shaded in orange (x): Unexpected plasmid replicon reported
Cells shaded in grey (/): participant did not submit plasmid replicons
NA: Not applicable; UN: Unexpected
[a] Expected but non-mandatory to report

3.6. 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 reported software was CGE ResFinder and its respective database. A full description of the methods reported by the participants is provided in Appendix 5.

In total, 114 sets of results were submitted regarding the detection of genetic determinants mediating AMR, by all participating laboratories. For *E. coli* (EURGen-2023-02) and *K. pneumoniae* (EURGen-2023-03), 29 participants submitted the results for the detection of AMR genes and PMs, while for *A. baumannii* (EURGen-2023-01) and *P. aeruginosa* (EURGen-2023-04), 28 participants submitted the results (Table 18, Figure 9).

Of the 114 sets of results submitted for detection of genetic determinants mediating AMR, 9.6% were fully correct (n=11). Additionally, in 61.4% of the sets of results (n=70), certain expected genetic determinants were missing, and in 74.6% of the submitted results (n=85), 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 (45.6% or n=52) (Table 18, Figure 9).

Overall, none of the participants reported correct expected genetic determinants of AMR for all the analysed strains, and none of the participants failed to identify all the expected determinants. Participants obtained between 11 and 33 points for the detection of genetic determinants of AMR. The participants obtained between 63.6% to 100% scores of their maximum possible scores (maximum possible score varies and depends upon the number of test strains analysed by the participant, see Table 13, Figure 6). The average concordance between expected and submitted results was 86% (Table 13, Figure 6).

Table 18. Distribution of submitted results regarding the detection of genetic determinants of AMR.

Test strain	Correct determinants	Only missing determinants	Only unexpected determinants	Missing+un-expected determinants	Total
EURGen-2023-01	2	5	7	14	**28**
EURGen-2023-02	2	8	4	15	**29**
EURGen-2023-03	5	4	16	4	**29**
EURGen-2023-04	2	1	6	19	**28**
Total	**11**	**18**	**33**	**52**	**114**

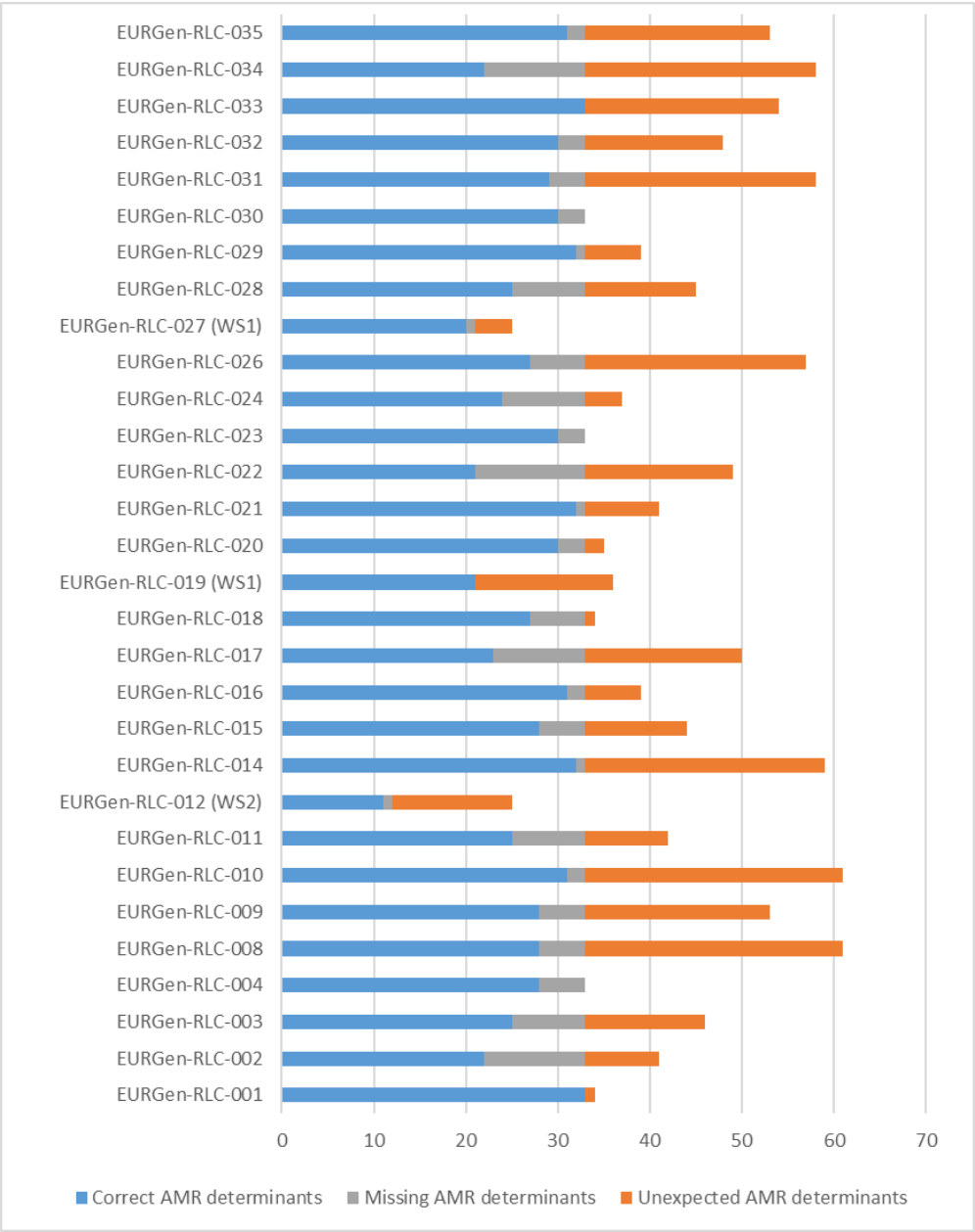

Figure 9. Distribution of submitted results regarding the detection of genetic determinants mediating AMR. WS1: Only submitted results for workstream 1 pathogens; WS2: Only submitted results for workstream 2 pathogens.

For strain EURGen-2023-01 (*A. baumannii*), participants were expected to detect four genes mediating AMR (*aph(3')-VI (*or *aph(3')-VIa), armA, bla*$_{NDM-1}$ and *bla*$_{OXA-23}$) and two chromosomal PMs (*gyrA* S81L and *parC* S84L). Three chromosomal PMs (*ftsI* A515V, *parC* V104I and *parC* D105E) were also expected to be detected in EURGen-2023-01 but were non-mandatory to report and yielded a "blank" score when reported in the webtool. In total, 28 laboratories submitted the detection of AMR determinants for this strain. All participating laboratories were able to detect the expected genes *armA*, *bla*$_{NDM-1}$ and *bla*$_{OXA-23}$. Most of the participants failed to detect the expected PMs *gyrA* S81L (n=16) and *parC* S84L (n=15). Of all 28 sets of submitted results for EURGen-2023-01, 21 participants reported at least one unexpected AMR determinant. The most reported unexpected AMR determinant was *bla*$_{OXA-66}$ (n=15), followed by *aph(3'')-Ib* (n=12), *bla*$_{ADC-25}$ (n=12), *aph(6)-Id* (n=12) and *tet(B)* (n=12). Only two sets of results contained all expected AMR

determinants, and no unexpected genes or chromosomal PMs were reported. Among the non-mandatory chromosomal PMs, most reported PM was *ftsI* A515V (n=9), followed by *parC* D105E (n=2) and *parC* V104I (n=2). 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 19.

Table 19. Results of the detection of genetic AMR determinants for each participant, for strain EURGen-2023-01 (*A. baumannii*)

	Expected									Unexpected													
Laboratories	*aph(3')-VI**	*armA*	*bla* NDM-1	*bla* OXA-23	*gyrA* S81L	*parC* S84L	*ftsI* A515V[a]	*parC* D105E[a]	*parC* V104I[a]	*aph(3'')-Ib*	*aph(6)-Ib*	*aph(3')-IV*	*bla* ADC-25	*bla* OXA-66	*aph(6)-Id*	*tet(B)*	*ampC* G247S	*ampC* N341T	*bla* OXA-82	*aph(3')-Ib*	**Correct (nr.)**	**Missing (nr.)**	**Unexpected (nr.)**	**Expected non-mandatory (nr.)**
EURGen-RLC-001	x	x	x	x	x	x	x	-	-												6	0	0	1
EURGen-RLC-002	-	x	x	x	-	-	-	-	-	x	x	x									3	3	3	0
EURGen-RLC-003	x	x	x	x	-	-	-	-	-	x			x	x	x						4	2	4	0
EURGen-RLC-004	x	x	x	x	x	x	x	-	-												6	0	0	1
EURGen-RLC-008	x	x	x	x	-	-	-	-	-	x			x	x	x	x					4	2	5	0
EURGen-RLC-009	x	x	x	x	-	-	-	-	-	x			x	x	x	x					4	2	5	0
EURGen-RLC-010	x	x	x	x	x	x	x	-	-	x			x	x	x	x					6	0	5	1
EURGen-RLC-011	x	x	x	x	-	-	-	-	-					x							4	2	1	0
EURGen-RLC-012	x	x	x	x	-	x	-	-	-	x			x	x	x	x	x	x			5	1	7	0
EURGen-RLC-014	x	x	x	x	x	x	x	-	-	x			x	x	x	x					6	0	5	1
EURGen-RLC-015	x	x	x	x	-	-	-	-	-												4	2	0	0
EURGen-RLC-016	x	x	x	x	x	x	-	x	x				x	x							6	0	2	2
EURGen-RLC-017	x	x	x	x	-	-	-	-	-							x			x		4	2	2	0
EURGen-RLC-018	x	x	x	x	-	-	-	-	-												4	2	0	0
EURGen-RLC-020	x	x	x	x	x	x	x	-	-					x							6	0	1	1
EURGen-RLC-021	x	x	x	x	x	x	x	-	-				x	x							6	0	2	1
EURGen-RLC-022	x	x	x	x	-	-	-	-	-											x	4	2	1	0
EURGen-RLC-023	x	x	x	x	-	-	-	-	-												4	2	0	0
EURGen-RLC-024	x	x	x	x	-	-	-	-	-												4	2	0	0
EURGen-RLC-026	x	x	x	x	-	-	-	-	-	x			x	x	x	x					4	2	5	0
EURGen-RLC-028	x	x	x	x	-	-	-	-	-						x	x					4	2	2	0
EURGen-RLC-029	x	x	x	x	x	x	x	-	-					x							6	0	1	1
EURGen-RLC-030	x	x	x	x	-	-	-	-	-												4	2	0	0
EURGen-RLC-031	-	x	x	x	x	x	-	-	-	x		x			x	x					5	1	4	0
EURGen-RLC-032	-	x	x	x	x	x	x	-	-	x		x			x						5	1	3	1
EURGen-RLC-033	x	x	x	x	x	x	x	-	-	x			x	x	x	x					6	0	5	1
EURGen-RLC-034	-	x	x	x	-	-	-	-	-	x		x	x	x	x	x					3	3	6	0
EURGen-RLC-035	-	x	x	x	x	x	-	x	x			x	x	x		x					5	1	4	2
EURGen-RLC-019	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	NA	NA	NA	NA
EURGen-RLC-027	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	NA	NA	NA	NA
Correct (nr.)	23	28	28	28	12	13	9	2	2	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	**Total**			
Missing or UN (nr.)	5	0	0	0	16	15	19	26	26	12	1	5	12	15	12	12	1	1	1	1	132	36	73	13

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
Cells shaded in grey (/): participant did not submit AMR genes and mutations
NA: Not applicable; UN: Unexpected
* Either *aph(3')-VI* or *aph(3')-VIa*
[a] Expected results but non-mandatory to report

For strain EURGen-2023-02 (*E. coli*), participants were expected to detect 12 genes (*aac(3)-IId*, *aac(6')-Ib-cr (*or *aac(6')-Ib-cr5)*, *bla*$_{\text{CMY-2}}$ (or *bla*$_{\text{CMY-59}}$), *bla*$_{\text{OXA-181}}$, *bla*$_{\text{NDM-5}}$, *bla*$_{\text{TEM-1}}$ (or *bla*$_{\text{TEM-1A}}$, *bla*$_{\text{TEM-1B}}$, *bla*$_{\text{TEM-1C}}$, *bla*$_{\text{TEM-1D}}$), *bla*$_{\text{CTX-M-15}}$, *qnrS1*, *sul1*, *sul2*, *dfrA12*, *dfrA17*), and five chromosomal PMs mediating AMR (*glpT* E448K, *gyrA* D87N, *gyrA* S83L, *parE* S458A and *parC* S80I). One AMR gene (*bla*$_{\text{OXA-1}}$), and two chromosomal mutations (*ftsI* N337NYRIN and *pmrB* Y358N) were also expected but were non-mandatory to report. In total, 29 laboratories submitted the detection of AMR determinants for this strain. The expected chromosomal mutation *glpT* E448K was not detected by 19 participants, while the expected PM *gyrA* D87N was not reported by five participants. Similarly, the expected AMR gene *aac(3)-IId* was not detected by five participants. The expected PMs *gyrA* S83L, *parE* S458A and *parC* S80I were not reported by four participants each. There were 16 more cases where expected AMR determinants were not reported. The total number of missing genetic determinants of AMR throughout all sets of submitted results was 57 (Table 20). The expected AMR genes *bla*$_{\text{CMY-2}}$ (or *bla*$_{\text{CMY-59}}$), *bla*$_{\text{NDM-5,}}$ *bla*$_{\text{OXA-18}}$, *bla*$_{\text{TEM-1}}$ (or *bla*$_{\text{TEM-1A}}$, *bla*$_{\text{TEM-1B}}$, *bla*$_{\text{TEM-1C}}$, *bla*$_{\text{TEM-1D}}$) were detected by all participants (n=29), while qnrS1 was reported by all except one participant (n=28). Overall, only two participants reported all expected results and no unexpected AMR determinants were reported. No set of results was missing all the expected determinants of AMR. Among the non-mandatory expected AMR determinants, the most reported mutation was *pmrB* Y358N (n=9), followed by *ftsI* N337NYRIN (n=6). The non-mandatory gene *bla*$_{\text{OXA-1}}$ was reported by 23 participants. Most of the laboratories reported unexpected genetic determinants of AMR (n=19). The most reported unexpected AMR gene was *tet(B)* (n=15), followed by *aph(3'')-Ib* (n=14), *aadA5* (n=13), *aph(6)-Id* (n=13) and *aadA2* (n=12). Four unexpected chromosomal PMs were detected for the strain EURGen-2023-02 but were not frequently reported (*gyrA* L83S (n=1), *gyrA* N87D (n=1), *ftsI* N33NYRIN (n=1), and *glpT* E488K (n=1)). The total number of unexpected genetic determinants of AMR throughout all sets of submitted results was 76 (Table 20).

Table 20. Results of the detection of genetic AMR determinants for each participant, for strain EURGen-2023-02 (*E. coli*)

	Expected																				Unexpected															
Laboratories	*aac(3)-IId*	*aac(6')-Ib-cr* *	*bla* CMY-2 *	*bla* CTX-M-15	*bla* NDM-5	*bla* OXA-181	*bla* TEM-1 *	*dfrA17*	*dfrA12*	*sul1*	*sul2*	*qnrS1*	*glpT* E448K	*gyrA* D87N	*gyrA* S83L	*parC* S80I	*parE* S458A	*bla* OXA-1 [a]	*ftsI* N337NYRIN[a]	*pmrB* Y358N[a]	*aadA2*	*aadA5*	*aph(3'')-Ib*	*aph(6)-Id*	*tet(B)*	*aac(3)-IIa*	*parC* S80L	*aac(6')-Ib*	*gyrA* L83S	*gyrA* N87D	*ftsI* N33NYRIN	*glpT* E488K	**Correct (nr.)**	**Missing (nr.)**	**Unexpected (nr.)**	**Expected non-mandatory (nr.)**
EURGen-RLC-001	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x													17	0	0	3
EURGen-RLC-002	x	x	x	x	x	x	x	x	x	x	x	x	-	-	-	-	-	x	-	-													12	5	0	1
EURGen-RLC-003	x	x	x	x	x	x	x	x	x	x	x	x	-	x	x	-	-	x	-	-	x	x	x	x									14	3	4	1
EURGen-RLC-004	x	x	x	x	x	x	x	x	x	x	x	x	x	-	-	x	-	x	x	-													14	3	0	2
EURGen-RLC-008	x	x	x	x	x	x	x	x	x	x	x	x	-	x	x	x	x	-	-	-	x	x	x	x	x								16	1	5	0
EURGen-RLC-009	x	x	x	x	x	x	x	x	x	x	x	x	-	x	x	x	x	x	-	-	x	x	x	x	x								16	1	5	1
EURGen-RLC-010	x	x	x	x	x	x	x	x	x	x	x	x	-	x	x	x	x	x	-	-	x	x	x	x	x								16	1	5	1
EURGen-RLC-011	-	x	x	x	x	x	x	x	-	x	x	x	-	-	x	-	x	-	-	-						x	x						12	5	2	0
EURGen-RLC-014	x	-	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x			x					16	1	6	3
EURGen-RLC-015	x	x	x	x	x	x	x	x	x	x	-	x	-	x	x	x	x	-	-	-													15	2	0	0
EURGen-RLC-016	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	-	-													17	0	0	1
EURGen-RLC-017	x	-	x	x	x	x	x	-	x	x	x	x	-	-	-	x	x	x	-	-					x				x	x			12	5	3	1
EURGen-RLC-018	x	x	x	x	x	x	x	x	x	x	x	x	-	x	x	x	x	x	-	-													16	1	0	1
EURGen-RLC-019	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	-	x	x	x	x	x	x								17	0	5	2
EURGen-RLC-020	x	x	x	x	x	x	x	-	x	x	x	-	x	x	x	x	x	x	-	x													15	2	0	2
EURGen-RLC-021	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	-	x											x		17	0	1	2
EURGen-RLC-022	-	x	x	-	x	x	x	x	-	-	-	x	-	x	x	x	x	-	-	-			x		x								11	6	2	0
EURGen-RLC-023	x	x	x	x	x	x	x	x	x	x	x	x	-	x	x	x	x	x	-	-													16	1	0	1
EURGen-RLC-024	x	x	x	x	x	x	x	x	x	x	x	x	-	-	x	-	-	x	-	-													13	4	0	1
EURGen-RLC-026	-	x	x	x	x	x	x	x	x	x	x	x	-	x	x	x	x	x	-	-	x	x	x	x	x	x							15	2	6	1
EURGen-RLC-027	x	x	x	x	x	x	x	x	x	x	x	x	-	x	x	x	x	x	-	-	x	x	x	x									16	1	4	1
EURGen-RLC-028	-	x	x	x	x	x	x	x	x	-	x	x	-	x	-	x	x	-	-	-		x	x	x	x								13	4	4	0
EURGen-RLC-029	x	-	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x					x			x					16	1	2	3
EURGen-RLC-030	x	x	x	x	x	x	x	x	x	x	x	x	-	x	x	x	x	x	-	x													16	1	0	2
EURGen-RLC-031	x	x	x	x	x	x	x	x	x	x	x	x	-	x	x	x	x	x	-	-	x	x	x	x	x								16	1	5	1
EURGen-RLC-032	x	x	x	x	x	x	x	x	x	x	x	x	-	x	x	x	x	x	x	x	x	x	x	x	x							x	16	1	6	3
EURGen-RLC-033	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x								17	0	5	3
EURGen-RLC-034	-	x	x	-	x	x	x	x	-	x	-	x	-	x	x	x	x	x	-	-			x	x	x								12	5	3	1
EURGen-RLC-035	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	-	-	-	x	x			x								17	0	3	0
EURGen-RLC-012	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	NA	NA	NA	NA
Correct (nr.)	24	26	29	27	29	29	29	27	26	27	26	28	10	24	25	25	25	23	6	9	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	**Totals**			
Missing or UN (nr.)	5	3	0	2	0	0	0	2	3	2	3	1	19	5	4	4	4	6	23	20	12	13	14	13	15	2	1	2	1	1	1	1	436	57	76	38

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
Cells shaded in grey (/): participant did not submit AMR genes and mutations
NA: Not applicable; UN: Unexpected
* Either *aac(6')-Ib-cr* or *aac(6')-Ib-cr5*, either *bla*CMY-2 or *bla*CMY-59, either *bla*TEM-1 or *bla*TEM-1A, *bla*TEM-1B, *bla*TEM-1C or *bla*TEM-1D
[a] Expected results but non-mandatory to report

For strain EURGen-2023-03 (*K. pneumoniae*), participants were expected to detect four genes mediating AMR (*bla*$_{SHV-1}$ (or *bla*$_{SHV-185}$, or *bla*$_{SHV-187}$), *bla*$_{TEM-1}$ (*bla*$_{TEM-1A}$, *bla*$_{TEM-1B}$, *bla*$_{TEM-1C}$, *bla*$_{TEM-1D}$), *bla*$_{CTX-M-3}$, *qnrS1*) and no chromosomal PMs were expected to be detected by the participants. However, detection of the mutation in *mgrB* due to the insertion sequence IS*1* (*mgrB::*IS*1*) which causes a disruption in the *mgrB* gene was expected but was non-mandatory to report. In total, 29 laboratories submitted the detection of AMR determinants for this strain. In six sets of submitted results, the expected gene *bla*$_{SHV-1}$ (or *bla*$_{SHV-185}$, or *bla*$_{SHV-187}$) was not reported, while in two sets of submitted results, the expected gene *qnrS1* was not reported. The total number of missing genetic determinants of AMR throughout all sets of submitted results was eight. Overall, five sets of results contained all expected genetic determinants, and no unexpected AMR genes and PMs were reported. No set of results was missing all the expected determinants of AMR. None of the participants reported the non-mandatory mutation *mgrB::*IS*1*. Most of the participants reported unexpected genetic determinants of AMR (n=21). The most reported unexpected AMR gene was *fosA* (n=19), followed by *oqxA* (n=16) and *oqxB* (n=16). The frequently reported unexpected chromosomal PMs were for the gene *acrR* (n=42), although others were also observed. The total number of unexpected genetic determinants of AMR throughout all sets of submitted results was 104 (Table 21).

Table 21. Results of the detection of genetic AMR determinants for each participant, for strain EURGen-2023-03 (*K. pneumoniae*)

Laboratories	*bla* CTX-M-3	*bla* SHV-1*	*bla* TEM-1*	*qnrS1*	*mgrB*::IS*1* [a]	*fosA*	*OqxA*	*OqxB*	*acrR*	*acrR* F172S	*acrR* F197I	*acrR* G164A	*acrR* K201M	*acrR* L195V	*acrR* P161R	*acrR* R173G	*gyrA*	*mgrB*	*parC*	*ramR*	*ramR* F18I	*ramR* L156V	*ramR* E175D	*uhpT* E350Q	Correct (nr.)	Missing (nr.)	Unexpected (nr.)	Expected non-mandatory (nr.)
EURGen-RLC-001	x	x	x	x	-																				4	0	0	0
EURGen-RLC-002	x	-	x	x	-	x	x	x																	3	1	3	0
EURGen-RLC-003	x	x	x	x	-	x	x	x																	4	0	3	0
EURGen-RLC-004	x	-	x	x	-																				3	1	0	0
EURGen-RLC-008	x	x	x	x	-	x	x	x		x	x	x	x	x	x	x									4	0	10	0
EURGen-RLC-009	x	x	x	x	-	x	x	x																	4	0	3	0
EURGen-RLC-010	x	x	x	x	-	x	x	x		x	x	x	x	x	x	x									4	0	10	0
EURGen-RLC-011	x	x	x	x	-	x	x	x	x																4	0	4	0
EURGen-RLC-014	x	x	x	x	-	x	x	x	x								x		x	x					4	0	7	0
EURGen-RLC-015	x	x	x	x	-	x				x	x	x	x	x	x	x									4	0	8	0
EURGen-RLC-016	x	x	x	x	-																				4	0	0	0
EURGen-RLC-017	x	x	x	x	-	x	x	x																	4	0	3	0
EURGen-RLC-018	x	-	x	x	-																				3	1	0	0
EURGen-RLC-019	x	x	x	x	-	x	x	x		x	x	x	x	x	x	x									4	0	10	0
EURGen-RLC-020	x	x	x	-	-																				3	1	0	0
EURGen-RLC-021	x	x	x	x	-	x	x	x																	4	0	3	0
EURGen-RLC-022	x	x	x	x	-	x	x	x													x	x	x		4	0	6	0
EURGen-RLC-023	x	x	x	x	-													x							4	0	1	0
EURGen-RLC-024	x	-	x	x	-	x											x								3	1	2	0
EURGen-RLC-026	x	x	x	-	-	x	x	x																	3	1	3	0
EURGen-RLC-027	x	x	x	x	-																				4	0	0	0
EURGen-RLC-028	x	-	x	x	-																				3	1	0	0
EURGen-RLC-029	x	x	x	x	-	x																			4	0	1	0
EURGen-RLC-030	x	x	x	x	-																				4	0	0	0
EURGen-RLC-031	x	-	x	x	-					x	x	x	x	x	x	x									3	1	7	0
EURGen-RLC-032	x	x	x	x	-	x	x	x																	4	0	3	0
EURGen-RLC-033	x	x	x	x	-	x	x	x																	4	0	3	0
EURGen-RLC-034	x	x	x	x	-	x	x	x			x	x	x	x	x										4	0	8	0
EURGen-RLC-035	x	x	x	x	-	x	x	x									x		x					x	4	0	6	0
EURGen-RLC-012	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	NA	NA	NA	NA
Correct (nr.)	29	23	29	27	0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA			**Total**	
Missing or UN (nr.)	0	6	0	2	29	19	16	16	2	5	6	6	6	6	6	5	3	1	2	1	1	1	1	1	108	8	104	0

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
Cells shaded in grey (/): participant did not submit AMR genes and mutations
NA: Not applicable; UN: Unexpected
*Either *bla*SHV-1 or *bla*SHV-18 or *bla*SHV-187), either *bla*TEM-1 or *bla*TEM-1A, *bla*TEM-1B, *bla*TEM-1C or *bla*TEM-1D)
[a] Expected results but non-mandatory to report

For strain EURGen-2023-04 (*P. aeruginosa*), participants were expected to detect five AMR genes (*aac(6')-Il, aac(3)-Id, bla*VIM-2*, bla*OXA-4*, crpP*) and one chromosomal PM (*gyrA* T83I). One PM (*parC* S87L) was also expected but not mandatory to report. In total, 28 laboratories submitted the detection of AMR determinants for this strain. Twelve participants missed the expected PM *gyrA* T83I, and the expected gene *bla*OXA-4 was not reported by eight participants. The expected gene *aac(6')-Il* was not reported by seven participants. There were eight 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 35. Overall, only two participants reported all expected genetic determinants, and no unexpected results were reported. The non-mandatory PM *parC* S87L was reported by 12 participants. Unexpected genetic determinants of AMR were reported by 25 laboratories. The most reported unexpected AMR gene was *bla*PAO (n=22),

followed by *fosA* (n=20), *dfrB5* (n=14), and *tet(G)* (n=13). The unexpected genes *bla*$_{OXA-486}$, *aph(3')-IIb* and *sul1* were reported by 12 participants each. The total number of unexpected genetic determinants of AMR throughout all sets of submitted results was 126 (Table 22).

Table 22. Results of the detection of genetic AMR determinants for each participant, for strain EURGen-2023-04 (*P. aeruginosa*)

	Expected							Unexpected																			
Laboratories	*aac(3)-Id*	*aac(6')-Il*	*bla* OXA-4	*bla* VIM-2	*crpP*	*gyrA* T83I	*parC* S87L[a]	*bla* OXA-486	*bla* OXA-494	*aadA2*	*aph(3')-IIb*	*bla* PAO	*sul1*	*dfrB5*	*fosA*	*tet(G)*	*aac(6')-Ii*	*bla* OXA-50	*bla* OXA-31	*bla* VIM-48	*gyrA* D87N	*parE* A473V	*aac(3)-Ib*	**Correct (nr.)**	**Missing (nr.)**	**Unexpected (nr.)**	**Expected non-mandatory (nr.)**
EURGen-RLC-001	x	x	x	x	x	x	x					x												6	0	1	1
EURGen-RLC-002	x	x	-	x	x	-	-					x			x									4	2	2	0
EURGen-RLC-003	-	x	-	x	x	-	-					x			x									3	3	2	0
EURGen-RLC-004	x	x	x	x	-	x	x																	5	1	0	1
EURGen-RLC-008	x	-	x	x	x	-	-	x		x	x	x	x	x	x	x								4	2	8	0
EURGen-RLC-009	x	x	-	x	x	-	-			x	x	x	x	x	x	x								4	2	7	0
EURGen-RLC-010	x	-	x	x	x	x	x	x		x		x	x	x	x	x	x							5	1	8	1
EURGen-RLC-011	x	x	x	x	x	-	-					x			x									5	1	2	0
EURGen-RLC-012	x	x	x	x	x	x	-	x		x	x	x		x		x								6	0	6	0
EURGen-RLC-014	x	x	x	x	x	x	x	x		x	x	x	x	x	x	x								6	0	8	1
EURGen-RLC-015	x	x	x	x	x	-	-					x		x	x									5	1	3	0
EURGen-RLC-016	x	-	x	x	-	x	-	x				x			x		x							4	2	4	0
EURGen-RLC-017	x	x	-	-	x	-	-				x	x	x	x	x	x		x	x	x				3	3	9	0
EURGen-RLC-018	x	x	-	x	x	-	-					x												4	2	1	0
EURGen-RLC-020	x	x	x	x	x	x	x	x																6	0	1	1
EURGen-RLC-021	x	x	-	x	x	x	x					x			x									5	1	2	1
EURGen-RLC-022	-	-	-	x	x	-	-	x			x	x	x	x	x	x								2	4	7	0
EURGen-RLC-023	x	x	x	x	x	x	x																	6	0	0	1
EURGen-RLC-024	x	x	-	x	x	-	-					x			x									4	2	2	0
EURGen-RLC-026	x	-	x	x	x	x	-	x	x	x	x	x	x	x	x	x	x							5	1	10	0
EURGen-RLC-028	x	x	x	x	x	-	-			x	x	x	x	x		x								5	1	6	0
EURGen-RLC-029	x	x	x	x	x	x	x	x							x									6	0	2	1
EURGen-RLC-030	x	x	x	x	x	x	x																	6	0	0	1
EURGen-RLC-031	x	-	x	x	x	x	x			x	x		x	x	x	x	x				x	x		5	1	9	1
EURGen-RLC-032	x	x	x	x	-	x	x			x	x				x									5	1	3	1
EURGen-RLC-033	x	x	x	x	x	x	x	x		x	x	x	x	x	x	x								6	0	8	1
EURGen-RLC-034	-	-	x	x	x	-	-	x			x	x	x	x	x	x							x	3	3	8	0
EURGen-RLC-035	-	x	x	x	x	x	-	x		x		x	x	x	x	x								5	1	7	0
EURGen-RLC-019	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	NA	NA	NA	NA
EURGen-RLC-027	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	NA	NA	NA	NA
Correct (nr.)	24	21	20	27	25	16	12	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA		Total		
Missing or UN (nr.)	4	7	8	1	3	12	16	12	1	11	12	21	12	14	20	13	4	1	1	1	1	1	1	133	35	126	12

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
Cells shaded in grey (/): participant did not submit AMR genes and mutations
NA: Not applicable; UN: Unexpected
[a] Expected results but non-mandatory to report

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, however, one participant (EURGen-RLC-020) reported two PMs in EURGen-2023-01 (C-42T and T-32A in the promoter region) and three PMs in EURGen-2023-04 strain (C-42T and T-32A in the promoter region, and upregulated *aAmpC*: -13G).

3.7. *In silico* prediction of antimicrobial resistance profiles

In silico prediction of AMR profiles was generally conducted simultaneously with the detection of the genetic determinants mediating AMR (Appendix 5).

In total, 28 participants submitted the results regarding the *in silico* prediction of AMR profiles. Two participants did not submit results for any test strain (EURGen-RLC-032 and EURGen-RLC-034). Two laboratories (EURGen-RLC-019 and EURGen-RLC-027) only submitted results for WS1 pathogens, while one participant (EURGen-RLC-012) submitted results for only WS2 pathogens. One participant (EURGen-RLC-035) did not submit results for the strain EURGen-2023-03 (*K. pneumoniae*). The remaining 24 participants submitted results for all four test strains in the EQA 2023.

Of the 105 AMR profiles that were submitted, 10.5% were fully correct (n=11). Additionally, in 63.8% of the submitted AMR profiles (n=67), certain expected antimicrobials were missing. Finally, in 55.2% of the submitted AMR profiles (n=58), 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 (29.5% or n=31) (Table 23, Figure 10).

Table 23. Distribution of submitted results regarding the *in silico* prediction of AMR profiles.

Test strain	Correct profiles	Only missing antimicrobials	Only unexpected antimicrobials	Missing+Un-expected antimicrobials	Total
EURGen-2023-01	4	9	1	12	**26**
EURGen-2023-02	4	21	0	2	**27**
EURGen-2023-03	2	2	18	4	**26**
EURGen-2023-04	1	4	8	13	**26**
Total	**11**	**36**	**27**	**31**	**105**

Overall, among the 28 participants that submitted the results, none of the participants correctly predicted all four expected AMR profiles, and 23 participants failed to correctly predict the expected profile of any test strain. Participants obtained points for the prediction of AMR profiles which corresponded to 37.2% to 100% of their maximum possible scores for the expected antimicrobials. The average concordance between expected and submitted antimicrobials was 87.2% (Table 13, Figure 6).

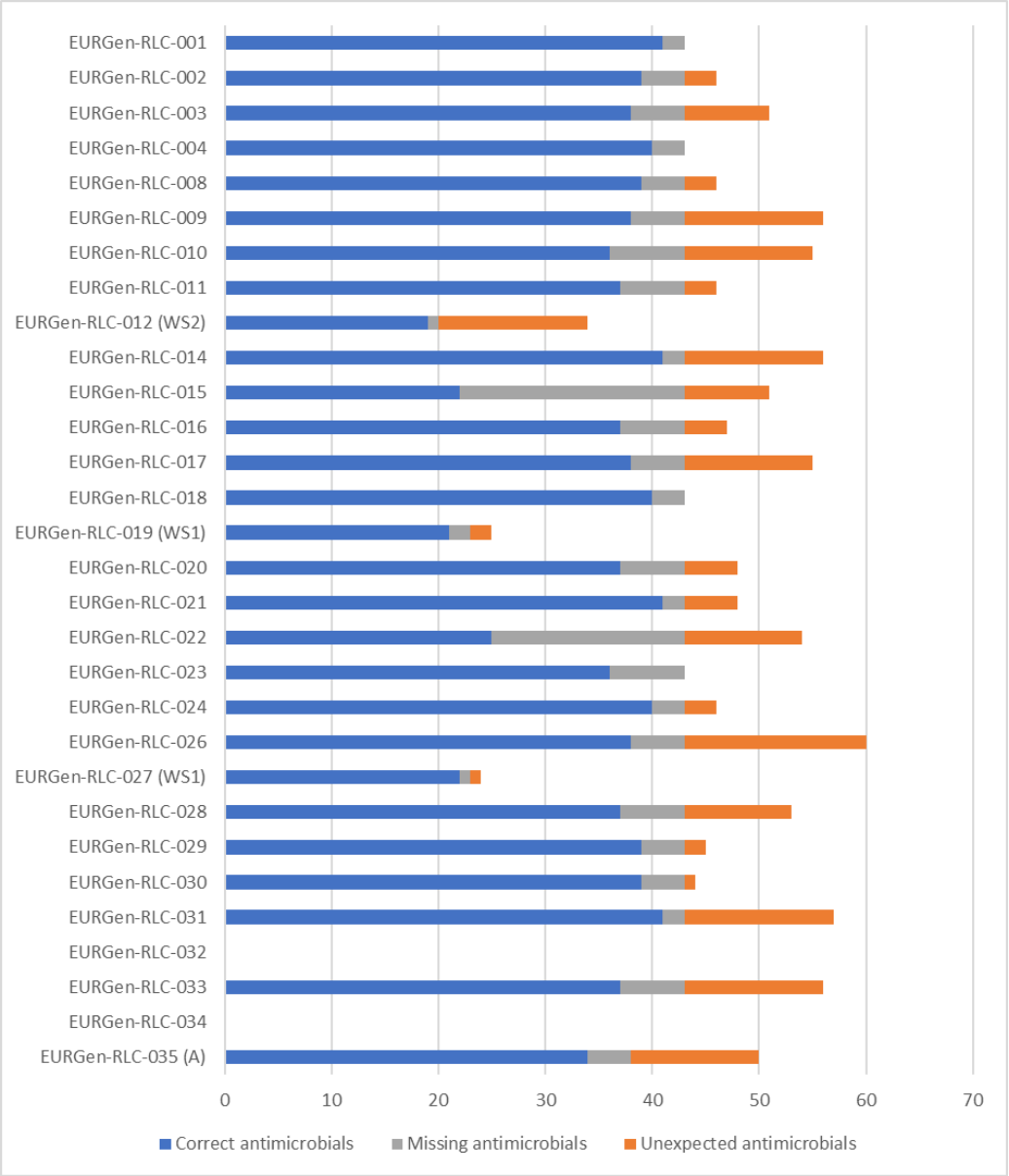

Figure 10. Distribution of submitted results regarding the *in silico* prediction of AMR profiles. WS1: Participants reported AMR profile for only workstream 1 pathogens; WS2: Participants reported AMR profile for workstream 2 pathogens; A: AMR profile for *K. pneumoniae* (EURGen-2023-03) was not submitted.

For strain EURGen-2023-01 (*A. baumannii*), participants were expected to predict resistance towards 10 antimicrobials (amikacin, ciprofloxacin, cefepime, ceftazidime, ceftazidime-avibactam, gentamicin, imipenem, meropenem, piperacillin-tazobactam, tobramycin). In total, 26 laboratories submitted the AMR profiles for this strain. The most common antimicrobial missing from the expected AMR profile was ciprofloxacin (n=19), followed by ceftazidime-avibactam (n=15). The expected antimicrobials cefepime, ceftazidime and piperacillin-tazobactam were not reported by three participants each. In two submitted AMR profiles, the expected antimicrobial tobramycin was not reported, and imipenem was not reported by one participant. The total number of missing antimicrobials throughout all submitted results was 46. Overall, four participants reported all expected antimicrobials correctly, and no unexpected antimicrobial was reported by these participants. Thirteen participants missed at least one expected antimicrobial (excluding

intrinsic resistance). Four participants missed only one antimicrobial while three participants were missing two antimicrobials. Overall, 46 antimicrobials were missing from all the submitted AMR profiles. There were no participants that missed all the expected antimicrobials. A total of 10 unexpected antimicrobials were reported by 16 participants, including two antimicrobials for which *A. baumannii* is intrinsically resistant (aztreonam and fosfomycin). The most reported unexpected antimicrobials were ampicillin (n=13) and amoxicillin-clavulanic acid (n=13), followed by cefotaxime (n=12), ertapenem (n=12), and aztreonam (n=6). The complete description of the concordances and discordances between the expected AMR profiles and 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-2023-01 (*A. baumannii*)

	Expected										Unexpected												
Laboratories	Amikacin	Cefepime	Ceftazidime	Ceftazidime-avibactam	Ciprofloxacin	Gentamicin	Imipenem	Meropenem	Piperacillin-tazobactam	Tobramycin	Ampicillin	Amoxicillin-clavulanic acid	Aztreonam[b]	Cefotaxime	Colistin	Ertapenem	Fosfomycin[b]	Sulfamethoxazole	Tigecycline	Trimethoprim	**Correct (nr.)**	**Missing (nr.)**	**Unexpected (nr.)**
EURGen-RLC-001	x	x	x	x	x	x	x	x	x	x											10	0	0
EURGen-RLC-002	x	x	x	-	-	x	x	x	x	x											8	2	0
EURGen-RLC-003	x	x	x	-	-	x	x	x	x	x	x	x		x		x					8	2	4
EURGen-RLC-004	x	x	x	x	x	x	x	x	x	x											10	0	0
EURGen-RLC-008	x	x	x	-	-	x	x	x	x	x											8	2	0
EURGen-RLC-009	x	x	x	-	-	x	x	x	x	x	x	x		x		x					8	2	4
EURGen-RLC-010	x	x	x	-	-	x	x	x	x	x	x	x		x		x					8	2	4
EURGen-RLC-011	x	x	x	x	-	x	x	x	x	x											9	1	0
EURGen-RLC-012	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x		x		x	10	0	7
EURGen-RLC-014	x	x	x	x	-	x	x	x	x	x	x	x		x		x					9	1	4
EURGen-RLC-015	x	-	-	-	-	x	-	x	-	-	x	x									3	7	2
EURGen-RLC-016	x	x	x	x	x	x	x	x	x	x			x				x				10	0	0
EURGen-RLC-017	x	x	x	-	-	x	x	x	x	x	x	x		x		x					8	2	4
EURGen-RLC-018	x	x	x	x	-	x	x	x	x	x											9	1	0
EURGen-RLC-020	x	x	x	-	x	x	x	x	-	-			x								7	3	0
EURGen-RLC-021	x	x	x	x	x	x	x	x	x	x			x								10	0	0
EURGen-RLC-022	x	-	-	-	-	x	x	x	x	x	x	x		x		x					6	4	4
EURGen-RLC-023	x	-	-	-	-	x	x	x	-	x											5	5	0
EURGen-RLC-024	x	x	x	x	-	x	x	x	x	x											9	1	0
EURGen-RLC-026	x	x	x	-	-	x	x	x	x	x	x	x		x		x					8	2	4
EURGen-RLC-028	x	x	x	-	-	x	x	x	x	x	x	x		x		x					8	2	4
EURGen-RLC-029	x	x	x	-	-	x	x	x	x	x											8	2	0
EURGen-RLC-030	x	x	x	x	-	x	x	x	x	x											9	1	0
EURGen-RLC-031	x	x	x	x	-	x	x	x	x	x	x	x	x	x		x			x		9	1	5
EURGen-RLC-033	x	x	x	-	-	x	x	x	x	x	x	x		x		x					8	2	4
EURGen-RLC-035	x	x	x	-	x	x	x	x	x	x	x	x	x	x		x					9	1	4
EURGen-RLC-019																					NA	NA	NA
EURGen-RLC-027																					NA	NA	NA
EURGen-RLC-032																					NA	NA	NA
EURGen-RLC-034																					NA	NA	NA
Correct (nr.)	26	23	23	11	7	26	25	26	23	24	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA		Total	
Missing or UN (nr.)	0	3	3	15	19	0	1	0	3	2	13	13	6	12	1	12	1	1	1	1	214	46	54

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
NA: Not applicable; UN: Unexpected
[b] Intrinsic resistance (based on EUCAST Expected Phenotypes Version 1, February 2022)

For strain EURGen-2023-02 (*E. coli*), participants were expected to predict resistance towards 18 antimicrobials (amikacin, amoxicillin-clavulanic acid, ampicillin, aztreonam, cefepime, cefotaxime, ceftazidime, ceftazidime-avibactam, ciprofloxacin, ertapenem, fosfomycin, gentamicin, imipenem, meropenem, piperacillin-tazobactam, sulfamethoxazole, tobramycin, trimethoprim). Colistin was expected in the AMR profile of this strain but was non-mandatory to report (accepted as a correct result but not a requirement for a fully correct AMR profile). In total, 26 laboratories submitted the AMR profiles for this strain. The expected antimicrobial fosfomycin was not reported by 21 participants, while ceftazidime-avibactam and aztreonam were missed by 16 and four participants, respectively. The expected antimicrobial gentamicin was not reported by three participants, while amikacin, cefepime and piperacillin-tazobactam were not reported by two participants each. There were six more cases of other missing antimicrobials. There were 56 total instances where expected antimicrobials were missing. Overall, four results contained all expected antimicrobials, and no unexpected antimicrobial was reported in these results. Eight participants were missing only one antimicrobial, and eight participants were missing two antimicrobials. None of the participants missed all the expected antimicrobials. Tigecycline was the only unexpected antimicrobial reported by two participants. The expected non-mandatory antimicrobial colistin was reported by only five participants. 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-2023-02 (*E. coli*)

	Expected																		Unexpected				
Laboratories	Amikacin	Amoxicillin-clavulanic acid	Ampicillin	Aztreonam	Cefepime	Cefotaxime	Ceftazidime	Ceftazidime-avibactam	Ciprofloxacin	Colistin[a]	Ertapenem	Fosfomycin	Gentamicin	Imipenem	Meropenem	Piperacillin-tazobactam	Sulfamethoxazole	Tobramycin	Trimethoprim	Tigecycline	**Correct (nr.)**	**Missing (nr.)**	**Unexpected (nr.)**	**Expected non-mandatory (nr.)**
EURGen-RLC-001	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x		18	0	0	1
EURGen-RLC-002	x	x	x	x	x	x	x	-	x	-	x	-	x	x	x	x	x	x	x		16	2	0	0
EURGen-RLC-003	x	x	x	x	x	x	x	-	x	-	x	-	x	x	x	x	x	x	x		16	2	0	0
EURGen-RLC-004	x	x	x	x	x	x	x	x	x	-	x	x	x	x	x	x	x	x	x		18	0	0	0
EURGen-RLC-008	x	x	x	x	x	x	x	-	x	-	x	-	x	x	x	x	x	x	x		16	2	0	0
EURGen-RLC-009	x	x	x	x	x	x	x	-	x	-	x	-	x	x	x	x	x	x	x		16	2	0	0
EURGen-RLC-010	-	x	x	x	x	x	x	-	x	-	x	-	x	x	x	x	x	x	x		15	3	0	0
EURGen-RLC-011	x	x	x	x	x	x	x	-	x	-	x	-	-	x	x	x	x	x	x		15	3	0	0
EURGen-RLC-014	x	x	x	x	x	x	x	x	x	-	x	-	x	x	x	x	x	x	x		17	1	0	0
EURGen-RLC-015	x	x	x	-	-	-	-	-	x	-	-	-	x	-	x	-	x	x	x		9	9	0	0
EURGen-RLC-016	x	x	x	x	x	x	x	x	x	-	x	x	x	x	x	x	x	x	x		18	0	0	0
EURGen-RLC-017	x	x	x	x	x	x	x	-	x	-	x	-	x	x	x	x	x	x	x		16	2	0	0
EURGen-RLC-018	x	x	x	x	x	x	x	x	x	-	x	-	x	x	x	x	x	x	x		17	1	0	0
EURGen-RLC-019	x	x	x	x	x	x	x	-	x	-	x	-	x	x	x	x	x	x	x		16	2	0	0
EURGen-RLC-020	x	x	x	x	x	x	x	-	x	x	x	x	x	x	x	x	x	x	x	x	17	1	1	1
EURGen-RLC-021	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x		18	0	0	1
EURGen-RLC-022	-	x	x	-	-	x	x	-	x	-	x	-	-	x	x	x	-	-	x		10	8	0	0
EURGen-RLC-023	x	x	x	x	x	x	x	x	x	-	x	-	x	x	x	x	x	x	x		17	1	0	0
EURGen-RLC-024	x	x	x	x	x	x	x	x	x	-	x	-	x	x	x	x	x	x	x		17	1	0	0
EURGen-RLC-026	x	x	x	x	x	x	x	-	x	-	x	-	x	x	x	x	x	x	x		16	2	0	0
EURGen-RLC-027	x	x	x	x	x	x	x	x	x	-	x	-	x	x	x	x	x	x	x		17	1	0	0
EURGen-RLC-028	x	x	x	-	x	x	x	-	x	-	x	-	-	x	x	x	x	x	x		14	4	0	0
EURGen-RLC-029	x	x	x	x	x	x	x	-	x	x	x	x	x	x	x	x	x	x	x		17	1	0	1
EURGen-RLC-030	x	x	x	x	x	x	x	x	x	x	x	-	x	x	x	x	x	x	x		17	1	0	1
EURGen-RLC-031	x	x	x	x	x	x	x	x	x	-	x	-	x	x	x	x	x	x	x	x	17	1	1	0
EURGen-RLC-033	x	x	x	-	x	x	x	-	x	-	x	-	x	x	x	x	x	x	x		15	3	0	0
EURGen-RLC-035	x	x	x	x	x	x	x	-	x	-	x	-	x	x	x	-	x	x	x		15	3	0	0
EURGen-RLC-012																					NA	NA	NA	NA
EURGen-RLC-032																					NA	NA	NA	NA
EURGen-RLC-034																					NA	NA	NA	NA
Correct (nr.)	25	27	27	23	25	26	26	11	27	5	26	6	24	26	27	25	26	26	27	NA		Total		
Missing or UN (nr.)	2	0	0	4	2	1	1	16	0	22	1	21	3	1	0	2	1	1	0	2	430	56	2	5

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
NA: Not applicable; UN: Unexpected
[a] Expected but non-mandatory to report

For strain EURGen-2023-03 (*K. pneumoniae*), participants were expected to predict resistance towards five antimicrobials (aztreonam, cefepime, cefotaxime, ceftazidime, ciprofloxacin). Additionally, the *in silico* prediction for colistin was expected for this test strain but non-mandatory to report (accepted as a correct result but not a requirement for a fully correct AMR profile). In total, 26 laboratories submitted the AMR profiles for this strain. The expected antimicrobial aztreonam was not reported by four participants, while ceftazidime and ciprofloxacin were not reported by two participants each. In total, there were 10 cases of antimicrobials missing from the AMR profiles of six participants. Two participants reported fully correct expected AMR profile and no unexpected antimicrobial was reported (excluding ampicillin). Moreover, four participants missed only one expected antimicrobial. Additionally, two participants reported the non-mandatory antimicrobial colistin in the submitted AMR profile. There were nine unexpected antimicrobials reported

by the participants, including ampicillin to which *K. pneumoniae* is intrinsically resistant. The participants that reported ampicillin were not penalized and were given a "blank" score instead. The most reported unexpected antimicrobial was ampicillin (n=24), followed by fosfomycin (n=18), trimethoprim (n=11), amoxicillin-clavulanic acid (n=5), piperacillin-tazobactam (n=4) and ertapenem (n=2). Most participants (n=22) reported one or more unexpected antimicrobials. The total number of unexpected antimicrobials throughout all submitted results was 43. 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-2023-03 (*K. pneumoniae*)

	Expected						Unexpected												
	Aztreonam	Cefepime	Cefotaxime	Ceftazidime	Ciprofloxacin	Colistin[a]	Ampicillin[b]	Amoxicillin-clavulanic acid	Ertapenem	Fosfomycin	Gentamicin	Imipenem	Meropenem	Piperacillin-tazobactam	Trimethoprim	**Correct (nr.)**	**Missing (nr.)**	**Unexpected (nr.)**	**Expected non-mandatory (nr.)**
EURGen-RLC-001	x	x	x	x	x	-	x									5	0	0	0
EURGen-RLC-002	x	x	x	x	x	-	x			x					x	5	0	2	0
EURGen-RLC-003	x	x	x	x	x	-				x					x	5	0	2	0
EURGen-RLC-004	x	x	x	-	x	-	x									4	1	0	0
EURGen-RLC-008	x	x	x	x	x	-	x			x					x	5	0	2	0
EURGen-RLC-009	x	x	x	x	x	-	x			x					x	5	0	2	0
EURGen-RLC-010	x	x	x	x	x	-	x			x						5	0	1	0
EURGen-RLC-011	x	x	x	x	-	-	x			x					x	4	1	2	0
EURGen-RLC-014	x	x	x	x	x	-	x			x	x				x	5	0	3	0
EURGen-RLC-015	-	-	-	-	x	-	x			x						1	4	1	0
EURGen-RLC-016	x	x	x	x	x	-	x	x	x					x		5	0	3	0
EURGen-RLC-017	-	x	x	x	x	-	x			x						4	1	1	0
EURGen-RLC-018	x	x	x	x	x	-	x									5	0	0	0
EURGen-RLC-019	x	x	x	x	x	-	x			x					x	5	0	2	0
EURGen-RLC-020	x	x	x	x	x	-	x	x						x		5	0	2	0
EURGen-RLC-021	-	x	x	x	-	-	x	x		x				x	x	3	2	4	0
EURGen-RLC-022	x	x	x	x	x	-	x			x					x	5	0	2	0
EURGen-RLC-023	-	x	x	x	x	x	x									4	1	0	1
EURGen-RLC-024	x	x	x	x	x	-	x			x						5	0	1	0
EURGen-RLC-026	x	x	x	x	x	-			x	x		x	x	x	x	5	0	6	0
EURGen-RLC-027	x	x	x	x	x	x	x	x								5	0	1	1
EURGen-RLC-028	x	x	x	x	x	-	x			x						5	0	1	0
EURGen-RLC-029	x	x	x	x	x	-	x			x						5	0	1	0
EURGen-RLC-030	x	x	x	x	x	-	x	x								5	0	1	0
EURGen-RLC-031	x	x	x	x	x	-	x			x						5	0	1	0
EURGen-RLC-033	x	x	x	x	x	-	x			x					x	5	0	2	0
EURGen-RLC-012																NA	NA	NA	NA
EURGen-RLC-032																NA	NA	NA	NA
EURGen-RLC-034																NA	NA	NA	NA
EURGen-RLC-035																NA	NA	NA	NA
Correct (nr.)	22	25	25	24	24	2	NA	NA	NA	NA	NA	NA	NA	NA	NA		Total		
Missing or UN (nr.)	4	1	1	2	2	24	24	5	2	18	1	1	1	4	11	120	10	43	2

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
NA: Not applicable; UN: Unexpected
[a] Expected but non-mandatory to report
[b] Intrinsic resistance (based on EUCAST Expected Phenotypes Version 1, February 2022)

For strain EURGen-2023-04 (*P. aeruginosa*), participants were expected to predict resistance towards 10 antimicrobials (amikacin, cefepime, ceftazidime, ceftazidime-avibactam, ciprofloxacin, gentamicin, imipenem, meropenem, piperacillin-tazobactam, tobramycin). In total, 26 laboratories submitted the AMR profiles for this strain. The most frequent antimicrobial missed by participants for this strain was ceftazidime-avibactam (n=15), followed by ciprofloxacin (n=3) and piperacillin-tazobactam (n=3). Other expected antimicrobials cefepime, ceftazidime, gentamicin and imipenem were not reported by two participants each. In total, the number of missing antimicrobials throughout all submitted AMR profiles was 32. Overall, only one result contained all expected antimicrobials. Three results were missing only one or two antimicrobials and none of the participants missed all the expected antimicrobials. Most of the participants reported unexpected antimicrobials (n=21). The total number of unexpected antimicrobials was nine and the most reported unexpected antimicrobial was fosfomycin (n=18), followed by ampicillin (n=12) and trimethoprim (n=12). Other unexpected antimicrobials include amoxicillin-clavulanic acid, cefotaxime, ertapenem and sulfamethoxazole, reported by 11 participants each. The total number of unexpected antimicrobials throughout all submitted results was 90. The complete description of the results submitted by participants is provided in Table 27.

Table 27. Results of the *in silico* prediction of AMR profiles for each participant, for strain EURGen-2023-04 (*P. aeruginosa*)

	Expected										Unexpected												
Laboratories	Amikacin	Cefepime	Ceftazidime	Ceftazidime-avibactam	Ciprofloxacin	Gentamicin	Imipenem	Meropenem	Piperacillin-tazobactam	Tobramycin	Amoxicillin-clavulanic acid	Ampicillin	Aztreonam	Cefotaxime	Ertapenem	Fosfomycin	Sulfamethoxazole	Tigecycline	Trimethoprim	**Correct (nr.)**	**Missing (nr.)**	**Unexpected (nr.)**
EURGen-RLC-001	x	x	x	x	x	x	x	x	x	x										10	0	0
EURGen-RLC-002	x	x	x	-	x	x	x	x	x	x						x				9	1	1
EURGen-RLC-003	x	x	x	-	x	-	x	x	x	x					x	x				8	2	2
EURGen-RLC-004	x	x	x	x	x	x	x	x	x	x										10	0	0
EURGen-RLC-008	x	x	x	-	x	x	x	x	x	x						x				9	1	1
EURGen-RLC-009	x	x	x	-	-	x	x	x	x	x	x	x		x	x	x	x		x	8	2	7
EURGen-RLC-010	x	x	x	-	x	x	x	x	x	x	x	x		x	x	x	x		x	9	1	7
EURGen-RLC-011	x	x	x	-	x	x	x	x	x	x						x				9	1	1
EURGen-RLC-012	x	x	x	x	x	x	x	x	x	x	x	x		x	x		x	x	x	10	0	7
EURGen-RLC-014	x	x	x	x	x	x	x	x	x	x	x	x		x	x	x			x	10	0	6
EURGen-RLC-015	-	-	-	-	x	x	-	x	-	-	x	x				x	x		x	3	7	5
EURGen-RLC-016	x	x	x	x	x	x	x	x	x	x						x				10	0	1
EURGen-RLC-017	x	x	x	-	-	x	x	x	x	x	x	x		x	x	x	x		x	8	2	7
EURGen-RLC-018	x	x	x	x	x	x	x	x	x	x										10	0	0
EURGen-RLC-020	x	-	-	-	x	x	-	-	-	x		x	x							4	6	2
EURGen-RLC-021	x	x	x	x	x	x	x	x	x	x						x				10	0	1
EURGen-RLC-022	x	x	x	-	x	x	x	x	x	x				x	x	x	x		x	9	1	5
EURGen-RLC-023	x	x	x	x	x	-	x	x	x	x										9	1	0
EURGen-RLC-024	x	x	x	x	x	x	x	x	x	x			x			x				10	0	2
EURGen-RLC-026	x	x	x	-	x	x	x	x	x	x	x	x		x	x	x	x		x	9	1	7
EURGen-RLC-028	x	x	x	-	-	x	x	x	x	x	x	x		x			x		x	8	2	5
EURGen-RLC-029	x	x	x	-	x	x	x	x	x	x						x				9	1	1
EURGen-RLC-030	x	x	x	x	x	x	x	x	x	x										10	0	0
EURGen-RLC-031	x	x	x	x	x	x	x	x	x	x	x	x		x	x	x	x		x	10	0	7
EURGen-RLC-033	x	x	x	-	x	x	x	x	x	x	x	x		x	x	x	x		x	9	1	7
EURGen-RLC-035	x	x	x	-	x	x	x	x	-	x	x	x		x	x	x	x	x	x	8	2	8
EURGen-RLC-019																				NA	NA	NA
EURGen-RLC-027																				NA	NA	NA
EURGen-RLC-032																				NA	NA	NA
EURGen-RLC-034																				NA	NA	NA
Correct (nr.)	25	24	24	11	23	24	24	25	23	25	NA	NA	NA	NA	NA	NA	NA	NA	NA		Total	
Missing or UN (nr.)	1	2	2	15	3	2	2	1	3	1	11	12	2	11	11	18	11	2	12	228	32	90

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
NA: Not applicable; UN: Unexpected

3.8. Feedback survey

The feedback survey was shared with the participants in October 2023. The deadline to submit the feedback was 6th November 2023, and the deadline was extended until 10th November 2023. By the extended deadline, three laboratories completed the feedback survey and submitted their comments. The participants were reminded again about the feedback survey during the webinar on 21st November 2023 and extended the deadline until 30th November 2023. The survey was open until 04th November 2023 to accept feedback from the participants, but no further feedback was received.

One participant chose usefulness of 10, while two other participants chose nine and six, respectively. Two participants answered that the preliminary individual EQA evaluation reports they received in October were clear and useful, while one participant answered that the reports were not clear and useful. Two participants answered that they took corrective actions based on the recommendations of the report while one answered that they did not take any action.

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 feedback received via email, and can be summarized in following main points with explanations:

- Changes should be made to the webtool to accept the submissions for the mutations caused by the insertion sequences.

- Some participants were less interested in prediction of AMR profiles from genomic data because that is not part of their routine analysis.

- One participant requested to consider revising the lists of antibiotics of clinical relevance which forms the basis for the *in silico* prediction of AMR profiles. The lists include antibiotics for which there is no clinical breakpoints for some species. In response to this, the EQA organizers believe that that the list of antimicrobials is for WS1 and WS2 pathogens in general, and some of the antimicrobials may not be relevant for the individual species included in these workstreams. This provides an opportunity for the participants to enhance their understanding and knowledge about the mechanisms of resistance, and to build the capacity for accurate detection and reporting of AMR determinants in these species.

- Some participants were unsure whether to report AMR determinants that might not confer clinical resistance on their own, such as quinolone resistance gene *qnrS1*. One participant requested to clarify this in the EQA protocol. Clinical resistance in bacteria is often achieved due to multiple resistance mechanisms. In response to this, the EQA organizers recommend reporting any acquired AMR determinant that contributes to increased resistance to a particular antimicrobial included in the list of relevant antimicrobials for that species. This will be highlighted in the protocols of upcoming EQAs.

4. DISCUSSION

4.1. Participation in the EQA

In the 2023 iteration of EURGen-RefLabCap EQA, 30 laboratories participated and completed the EQA (76.9% of the 39 invited laboratories). These represented 29 of the 37 countries involved in the project (78.4%). This was an improvement from the 2022 EQA in which laboratories from 27 countries participated. Some laboratories that did not sign up for the 2023 EQA have not yet implemented WGS for the analysis of CRE/CCRE, C/CRPa and C/CRAb. Furthermore, some laboratories were having time and personnel constraints, therefore might be able to participate in future EQAs.

4.2. Quality control of submitted sequences

In the 2023 EQA, participants were also invited to submit the sequences they generated for quality control analysis. Most of the submitted Illumina sequences showed sufficient quality for the type of analysis performed. Overall, quality issues were evenly found in all the bacterial species. Two laboratories achieved relatively low score between 75% to 80% of their maximum possible score. However, this lack of quality did not significantly affect the ability of these laboratories to satisfactorily perform bioinformatic analysis and they achieved an overall score of 92% to 96% of their maximum possible score for the bioinformatics analyses. The sequences from one laboratory (EURGen-RLC-012) were significantly below the quality thresholds and achieved an overall score of 35% of the maximum possible score. Consequently, this laboratory achieved the lowest score of 58.6% of the maximum possible score for the bioinformatics analysis. A further explanation of the issues observed during the QC of the sequences were provided to individual laboratories to help improve their quality of the analyses. This highlights the importance of ensuring good quality sequencing data to accurately detect the genetic AMR determinants and predict the associated AMR profiles.

For the long-read sequences, the main challenge to routine sequencing using Oxford Nanopore technologies was the sequencing yield. Based on recommendations of 30x coverage, it was clearly a challenge for participants to reliably achieve enough throughput, with one participant having issues across multiple isolates. Because of the low yield, achieving a reliable coverage across plasmids is not always possible, as all participants had lower coverage on plasmids, and not sufficient to achieve 20x coverage of all plasmids across multiple samples. As plasmids are frequently involved in dissemination of AMR genes, this is regarded as a major concern for the use of these sequences for AMR surveillance.

4.3. Prediction of sequence types

The prediction of ST for *E. coli, K. pneumoniae,* and *P. aeruginosa* was in full concordance with the expected results. However, there were 11 discordances reported for the *A. baumannii* strain EURGen-2023-01. Most of these discordances (n=7) were due to the use of Pasteur MLST scheme for *A. baumannii* by the participants, as opposed to the Oxford scheme which was proposed for this EQA in the protocol.

Some of the discordances were due to the presence of multicopy *gdhB* locus i.e., *gdhB*_3 and *gdhB*_189 alleles for this strain, resulting in two sequence types i.e., ST136 and ST1851 for the strain EURGen-2023-01. This was also detected while preparing the expected results when using the Oxford MLST scheme for *A. baumannii*. The sequence type with lowest number (i.e., ST136) was selected for the expected results and the use of same strategy for reporting ST was proposed in the EQA protocol.

For the self-evaluation, it should be considered that these discrepancies do not represent a flaw in the bioinformatics analysis performed by the participants but were due to not following the guidelines for the analysis and reporting of the results as mentioned in the EQA 2023 protocol. It is important to understand that the bioinformatics capacity and knowledge required for using either MLST scheme is the same, and participants should adhere to analysis and reporting rules as recommended for the EQA. Additionally, EQA providers do not recommend any MLST scheme for the participants' routine analysis outside the EQA activity and participants should adhere to their local guidelines.

4.4. Detection of plasmid replicon types

For the detection of plasmid replicons, 28.6% of submitted results were missing certain expected replicon genes. Among the expected replicons, most commonly missed replicons were ColKP3 and repB(R1701), which were missing in eight sets of participants each. 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, the choice of thresholds to generate expected results was arbitrary and the use of different thresholds is not necessarily incorrect. Additionally, the reason for missing replicons might be due to the use of different bioinformatics tools or the databases versions than the ones used for generating expected results. For example, some of the participants that missed certain expected results were using Ridom SeqSphere+[32] (Appendix 4). These programs use different algorithms and databases for the detection of replicons and may not be able to detect certain replicon genes or may also detect other replicons which are not part of the expected results.

In the expected results of EQA 2023, certain replicons were considered as non-mandatory to report. This was due to the fact that these replicons could only be detected in one set of expected results produced at DTU and SSI, or only detected in the long-read sequencing data. The non-mandatory replicons were Col(pHAD28) and IncQ1 in EURGen-2023-02 and Col(pHAD28) in EURGen-2023-03. The most commonly missed non-mandatory replicon was Col(pHAD28) which was missing in 39 out of 56 sets of submitted results. While preparing the expected results, none of the non-mandatory replicons were detected in the FASTA and FASTQ files shared with the participants, thus it is not unforeseen that these replicons were also missed by most of the participants that used these test materials. This difference in expected results furthermore strengthens 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 (because 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 participants reported unexpected replicon in 23.3% sets of results (n=14), less frequently than the missing expected replicons. Some of these discordances might be due to reporting of several similar replicons appeared in the output. This might be due to the lack of knowledge of difference between similar replicon types or insufficient scrutiny of the results from the bioinformatics tools. For example, the reported unexpected replicon ColpVC was not detected while preparing the expected results (using thresholds of 90% for both coverage and identity). The participants that reported ColpVC might have used thresholds lower than 90% for coverage and identity. Furthermore, the replicon ColpVC has a fairly high nucleotide identity (95.35%) with the expected replicon Col(BS512), and

[32] https://www.ridom.de/seqsphere/

participants might have reported both of these replicons. A more careful analysis of the results is needed in such a way that only the replicon with highest percentage of identity must be reported, while other replicons for the same location must then be discarded.

4.5. Detection of genes and chromosomal point mutations mediating AMR

For the detection of genetic AMR determinants, more than 60% of submitted sets of results were incomplete and were missing one or more expected genetic AMR determinants. Most of these missing determinants were chromosomal mutations conferring AMR. For example, the PMs *gyrA* S81L and *parC* S84L in *A. baumannii* strain EURGen-2023-01 were missing in 16 and 15 sets of results, respectively. Similarly, the point mutation *gyrA* T83I in *P. aeruginosa* strain EURGen-2023-04 was missed by 12 participants. This might be due to the lack of database for the chromosomal mutations for these species: in the ResFinder tool, the supporting PMs database (PointFinder) does not contain PMs for *A. baumannii* and *P. aeruginosa*. For generating the expected results, consensus results from AMRFinder+ (AMRFinder+ database) and RGI (with CARD database) were used for the PMs and most participants that used either of these databases were able to detect the expected PMs in *A. baumannii* and *P. aeruginosa.* Surprisingly, there were still two laboratories that used either CARD or AMRFinder+ databases and did not report the expected PMs in those species. This might be due to the use of local pipelines and algorithms by these laboratories for the detection of AMR determinants, or due to the use of different versions of the databases than those used to prepare the expected results.

Similarly, another common PM that was missing was *glpT* E448K in *E. coli* EURGen-2023-02, conferring resistance to fosfomycin. There were 19 participants that did not report this PM. Most of the participants used ResFinder (with associated ResFinder and PointFinder databases) and *glpT* mutations are not included for *E. coli* in these databases. It is important to understand that clinical resistance against an antimicrobial agent is often due to the combination of multiple resistance mechanisms, and multiple chromosomal PMs may have a cumulative effect, thus it is especially important to obtain a complete profile of these mutations. Therefore, participants may consider using confirmatory bioinformatics tools and databases for the detection of AMR determinants to obtain a complete genetic AMR profile.

In addition to the missing PMs, expected AMR genes were also missing. The most commonly missing AMR genes belonged to the group of aminoglycoside resistance genes (n=24 times). Specifically, the *aph(3')-VI* (or *aph(3')-VIa*) was missed five times in EURGen-2023-01, while *aac(3)-IId* and *aac(6')-Ib-cr* (or *aac(6')-Ib-cr5*) were missed five times and three times in EURGen-2023-02, respectively. Moreover, *aac(6')-Il* was missed seven times and *aac(3)-Id* was missed four times in EURGen-2023-04. The following most commonly missed genes were those encoding β-lactamases (n=17 times). Specifically, *bla*$_{\text{CTX-M-15}}$ in strain EURGen-2023-02 was missed two times, *bla*$_{\text{SHV-1}}$ (or *bla*$_{\text{SHV-185}}$, or *bla*$_{\text{SHV-187}}$) in strain EURGen-2023-03 was missed six times. Furthermore, *bla*$_{\text{OXA-4}}$ was missed seven times while *bla*$_{\text{VIM-2}}$ was missed once in EURGen-2023-04. Moreover, the genes encoding the enzymes that inhibit folate synthesis were missed 10 times (*dfrA12* (n=2); *dfrA17* (n=3); *sul1* (n=2); *sul2* (n=3)), while quinolone resistance genes were missed six times (*crpP* (n=3); *qnrS1* (n=3)).

The proportion of missing expected AMR determinants in *E. coli and K. pneumoniae* was considerably lower in EQA 2023 (10.7%) than the proportion observed in the 2022 EQA (16.7%). One reason is that in the current EQA, most of the discrepancies that exist in the databases were considered while preparing the expected results. For example, *bla*$_{\text{SHV-1}}$ has a high sequence similarity with *bla*$_{\text{SHV-185}}$ and *bla*$_{\text{SHV-187}}$ (>99%) and depending on the bioinformatics tools used, any of these genes might be detected and reported by the participants. Another example of discrepancy of the databases is that the ResFinder

reports aminoglycoside resistance gene *aac(6')-Ib-cr* while CARD and AMRFinder+ report the same region as *aac(6')-Ib-cr5*. To compensate for these discrepancies and to ensure correct scoring, the webtool in 2023 EQA was designed to accept any of these genes as correct answers.

There were certain situations where the expected AMR determinant was not mandatory for the participants to report, and only a few participants were able to detect these determinants. These situations arose while preparing the expected results for the EQA 2023, in the cases when there was no consensus between sets of results obtained from different tools. For example, in EURGen-2023-01, chromosomal PMs *parC* V104I and *parC* D105E, that confer high-level quinolone resistance in *A. baumannii,* were only detected with RGI (with CARD database), while the PM *ftsI* A515V, that contributes to the increased resistance to carbapenems in *A. baumannii,* was only detected with AMRFinder+. The chromosomal PM *ftsI* A515V was reported by nine participants, while PMs *parC* V104I and *parC* D105E were reported by only two participants each. Similar situations were detected in the remaining three test strains included in this EQA. Most of the participants failed to detect *ftsI* N337NYRIN and *pmrB* Y358N AMR mutations in EURGen-2023-02, and *parC* S86L in EURGen-2023-04.

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. A permanent solution could be to ensure communication between curators of the most widely used databases, and to opt to use sequences and nomenclature that are part of reference sequence databases such as NCBI RefSeq. Also, consolidation and synchronization of the databases before the release of new database versions might be helpful to eliminate these discrepancies. Furthermore, 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 detail to allow this type of retroactive investigation (Appendices 3, 4 and 5), and they are encouraged to improve their data and metadata registry and reporting processes.

Additionally, in some of the situations described previously, and others, there were presumable spelling, distraction, or submission mistakes, such as selection of *aph(3')-IV,* specifically by the participants that missed the expected AMR gene *aph(3')-VI* for strain EURGen-2023-01. The reporting of mutations *ftsI* N33NYRIN and *glpT* E488K instead of the expected mutations *ftsI* N337NYRIN and *glpT* E448K, respectively, in EURGen-2023-02 were seemingly due to spelling mistakes. There were also situations where PMs in *gyrA*, *parC* and *ramR* (although not expected) in EURGen-2023-03 were seemingly intended to be reported, but participants never defined the specific mutation to report, only the target gene. It is important to report the AMR gene or PM correctly and carefully, since reporting an unexpected genetic determinant can affect the prediction of antimicrobial susceptibility profile and considering that many of the analyses can be performed automatically by algorithms that cannot process spelling variations. Therefore, participants should ensure a more attentive review and recording of results while working with these data.

In addition to the missing AMR determinants, another common issue was the reporting of unexpected genetic AMR determinants by the participants, with 75% of sets of results containing unexpected AMR determinants. In some situations, this was due to insufficient knowledge regarding the impact of certain genes or PMs in the expected resistance profiles of the species included in this EQA. Some participants have reported genes for species in which they are intrinsic. For example, 15 participants reported $bla_{\text{ADC-25}}$ and 12 participants reported $bla_{\text{OXA-66}}$ in *A. baumannii* strain EURGen-2023-01. Although these β-lactamases are present in this strain, they are intrinsic in *A. baumannii* and do not contribute to a decrease in the susceptibility to β-lactam antibiotics included in this EQA. Moreover,

intrinsic resistance genes in *K. pneumoniae* were incorrectly reported for EURGen-2023-03, specifically *fosA* (n=19), *oqxA* (n=16) and *oqxB* (n=16). This was also observed in the previous iteration of the EQA where these three intrinsic genes were frequently reported by the participants in *K. pneumoniae* strains. For *P. aeruginosa* strain EURGen-2023-04, intrinsic resistance genes were also frequently reported. These included β-lactamase encoding genes *bla*$_{PAO}$ (n=21), and *bla*$_{OXA-50}$ like genes, *bla*$_{OXA-486}$ (n=12) and *bla*$_{OXA-494}$ (n=1). Participants also reported the fosfomycin resistance gene *fosA* (n=20), which is intrinsic in *P. aeruginosa*. In some cases, participants reported chromosomal PMs in *gyrA*, *parC*, *and parE* that, albeit present, have not been proven to be associated with decreased susceptibility towards quinolones. Similarly, several mutations were also reported in the target genes *acrR* and *ramR*, and in the same way these have an unconfirmed impact on the AMR profiles of the isolates.

Most of the remaining unexpectedly reported genetic determinants were due to the misinterpretation of the EQA 2023 protocol. Some participants have frequently reported acquired AMR genes and PMs which are present in the EQA test strains, however, the antimicrobials that they confer resistance towards were not part of the EQA 2023. For example, participants reported genes conferring resistance to antimicrobials not included in this EQA such as tetracycline (*tet(B), tet(G)*), streptomycin (*aadA2, aadA5, aph(6)-Id, aph(3'')-Ib*) and kanamycin (*aph(3')-IIb*). Several participants seemingly reported all genetic determinants detected by the bioinformatics tools without carefully examining the data. Many bioinformatics tools include the information about the location of the gene on the sequence being analysed, i.e., the information about the contig number (if using draft assemblies), start and end locations within that contig, and size of the gene. 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. Often, all the variants which have a high sequence similarity are reported by the tools, such as *bla*$_{TEM}$ variants when using ResFinder. It was reassuring that none of the participants reported more than one variant of *bla*$_{TEM-1}$, as opposed to what occurred in the previous EQA in 2022.

Although it is instinctive to report all the AMR determinants found in order not to miss any determinant that might lead to AMR, this strategy might affect the interpretation and comparability of results between different settings. These situations emphasize that laboratories should take a judicious and critical approach while reporting the genetic determinants of AMR and become familiar with the underlying genetic mechanisms of resistance that are relevant for the different species analysed in their settings. 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.

4.6. *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=46, in strains EURGen-2023-01, EURGen-2023-02 and EURGen-2023-03), and ciprofloxacin (n= 24, in strains EURGen-2023-01, EURGen-2023-03, and EURGen-2023-04). Furthermore, another common antimicrobial which was missed by the majority of the participants was fosfomycin (n=21, in strain EURGen-2023-02).

The absence of ceftazidime-avibactam 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 carbapenemase genes *bla*$_{\text{NDM-1}}$, *bla*$_{\text{NDM-5}}$, *bla*$_{\text{OXA-23}}$, and *bla*$_{\text{VIM-2}}$ which were part of the expected results (which is an error and increased the difficulty of this prediction, especially because the antimicrobial exists in the database associated with other genes).

In case of the missing expected antimicrobial ciprofloxacin in *A. baumannii*, the problem arises due to the lack of database of PMs conferring AMR for this species while using ResFinder (supported by the PointFinder database). In *A. baumannii*, mutation-based ciprofloxacin resistance tends to emerge primarily due to the PM *gyrA* S81L, followed by secondary mutations in the *parC* S84L, which were part of the expected results for the *A. baumannii* strain EURGen-2023-01. These expected results were prepared using three different bioinformatics tools and associated databases i.e., ResfFinder, AMRFinder+, and RGI. Similarly, the chromosomal PM *glpT* E448K conferring fosfomycin resistance is not part of the PointFinder database, hence it was missing in most of the submitted AMR profiles for the *E. coli* strain EURGen-2023-02.

In addition to understanding the genetic and molecular mechanisms of resistance, 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. Moreover, these issues 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 problem of reporting unexpected antimicrobials was more prevalent than the missing antimicrobials during the EQA 2023 and all participants have reported unexpected antimicrobials. There was a total of 15 unexpected antimicrobials reported by the participants for all four strains. Most of these problems were observed in the *A. baumannii* and *P. aeruginosa* strains and were mainly due to the reporting of antimicrobials for which the strain is intrinsically resistant. In *A. baumannii* and *P. aeruginosa* strains (EURGen-2023-01 and EURGen-2023-04), the β-lactams i.e., ampicillin, amoxicillin-clavulanic acid, cefotaxime and ertapenem, were the most reported unexpected antimicrobials. Although these strains are resistant to these antimicrobials, resistance is due to the combination of intrinsic mechanisms such as the presence of chromosomal cephalosporinases and carbapenemases (*bla*$_{\text{ADC}}$ and *bla*$_{\text{OXA-51}}$-type), and low permeability to certain antimicrobials (e.g., ertapenem). Therefore, following the guidelines in the EQA 2023 protocol regarding the reporting of intrinsic resistance mechanisms, these antimicrobials should not have been reported for *A. baumannii* and *P. aeruginosa*. Similarly, the incorrect reporting of resistance towards fosfomycin, as discussed previously in relation to the detection of genetic determinants of AMR, are direct consequences of detection of intrinsic *fosA* gene, not proven to be associated with decreased susceptibility towards fosfomycin in these species. Therefore, neither the genetic determinants nor the AMR profiles should be part of submitted results.

Finally, for resistance to antimicrobials that were expected but non-mandatory results, very few participants predicted colistin resistance in strain EURGen-2023-02 and EURGen-2023-03. In EURGen-2023-02, colistin resistance was mediated by the chromosomal PM *pmrB* Y358N (non-mandatory to report), while in EURGen-2023-03, the colistin resistance was due to the insertion sequence IS*1* causing truncation of the *mgrB* gene. Since the EQA 2023 webtool was designed to report point mutations by their amino-acid substitutions and it was not possible to report mutations caused by insertions, none of the participants could report *mgrB*::IS*1.* Two participants communicated the problem to the EQA organizers, which will be addressed in the next iteration of the EQA. Moreover, these

mutations (and thus, the respective associated AMR profile) are 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. The participants should be familiar with the pros and cons of each bioinformatics tool and use more than one bioinformatics tools and databases to confirm the presence and the absence of the genetic determinants.

4.7. Addressing the feedback from the participants

The feedback from the participants was used to implement updates to the evaluation of the EQA (i.e., adjustments to scoring in the webtool), to update the individual evaluation reports, to update this present aggregated report and to produce certificates of participation.

The feedback might furthermore guide updates to future EQAs, specifically:

- The main change in the webtool for the submission of the results is to make it possible for the participants to report mutations caused by insertion sequences. An explanation on how to report such mutations will be added to the protocol of upcoming EQAs;

- The main change in the protocol of upcoming EQAs refers to a more detailed explanation of what genes and chromosomal PMs the participants are expected to report (e.g. should the intrinsic AMR genes be reported, or not), as well as the AMR profiles that should be reported (e.g. should the intrinsic AMR profiles be reported, or not,). One example of this is the gene *bla*$_{\text{SHV-1}}$ (and variants) in EURGen-2023-03 (*K. pneumoniae*) which is part of the expected results for this strain. The recent literature and the resistome analysis of publicly available *K. pneumoniae* suggests that the *bla*$_{\text{SHV-1}}$ is intrinsic in *K. pneumoniae,* thus it will not be included in the expected results for *K. pneumoniae* in future EQAs. This shows that our understanding of the mechanisms of AMR is evolving, and it is important to be informed about the new knowledge and trends in the field of AMR.

5. CONCLUSION AND RECOMMENDATIONS

The results from the EURGen-RefLabCap 2023 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 bioinformatics tools and databases used. Many of the discrepancies are due to the lack of tools and databases for the detection of PMs associated with antimicrobial resistance in *A. baumannii* and *P. aeruginosa*. Most of these discrepancies can be alleviated by using multiple bioinformatics tools and databases for the detection of AMR determinants. However, these discrepancies should not be interpreted as a lack of knowledge and bioinformatics capacity by the participants, but instead they underscore 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 widely used bioinformatics tools and databases should try to improve the databases and include the PMs conferring AMR in *P. aeruginosa* and *A. baumannii;*
- Curators of bioinformatics tools and databases should engage in ongoing, active dialogue to ensure conformity between approaches;
- Curators of bioinformatics tools and databases should strive for the harmonization and synchronization of nomenclature and databases of AMR determinants;
- 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 communicate their suggestions, strange observations, and potential problems to the curators of bioinformatics tools and databases.

In addition to the discrepancies caused by the differences in the bioinformatics tools and databases used, there were significant numbers of discordances due to misinterpretation of the EQA protocol and/or insufficient knowledge about certain genetic mechanisms involved in AMR, leading to the reporting of unexpected AMR genes and chromosomal PMs, as well as the prediction of associated resistance towards unexpected antimicrobials. This issue was mainly prevalent in the results reported for *A. baumannii* and *P. aeruginosa*, and to some extent, also for *K. pneumoniae*. Moreover, there were situations 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 associated with the insufficient knowledge about certain mechanisms of resistance in the above-mentioned species. 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 ensure the use of multiple bioinformatics tools and databases for the detection of genetic determinants since bioinformatics tools and databases can be limited to the analysis of only a few bacterial species which contributes to the false-negative results;
- Laboratories should be familiar with the bioinformatics tools they use, and the contents of the respective databases;
- 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 2023 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: The quality control parameters included for the evaluation of Illumina sequences submitted by the participants in EQA 2023.

Parameters	**Description**
Number of reads	The number of reads refers to the sequence yield, how much was sequenced.
Number of reads after trimming	The number of reads remaining after quality trimming and common adapter removal.
Q-score R1/R2	Average quality score of the bases in the forward /reverse reads, treated separately.
Number of reads mapped to the reference chromosome	The number of reads, which map directly to the chromosome of the closed reference genome.
Number of reads mapped to plasmid N (if any)	The number of reads, which map directly to each specified plasmid of the closed reference genome.
Number of reads mapped to the complete genome	The number of reads, which map directly to the closed reference genome.
Proportion of reads mapped to the reference DNA sequence (%)	The proportion of reads, which map directly to the closed reference genome. This cannot exceed 100%.
Coverage of the reference genome/chromosome/plasmid N (%)	The extent to which reads have covered the entirety reference genome/chromosome/plasmid N. This cannot exceed 100%.
Coverage 5/10/20x of genome/chromosome/plasmid N (%)	The coverage of minimum depth X of each genomic element. This cannot exceed 100%.
Depth of coverage: Complete genome/chromosome/plasmid N	Number of base pairs sequenced divided by the total size of the closed reference genome/chromosome/plasmid N. This number can be rounded to the nearest integer. In essence, this number describes the number of times the sequenced base pairs cover the reference DNA and is often ended with an "x" (e.g. 30x).
Average insert size	The average length of DNA between the adapters. (only calculated for paired-end sequencing)
Size of assembled genome	The total size of all contigs in base pairs.
Size of assembled genome (200bp)	The total size of all contigs in base pairs, only counting contigs more than 200bp
Size of assembled genome per total size of DNA sequence (%)	Size of assembly compared to the size of the reference genome. Should be as close to 100 % as possible.
Size of assembled genome per total size of DNA sequence (contigs above 200bp) (%)	Size of assembly compared to the size of the reference genome, only counting contigs more than 200bp. Should be as close to 100 % as possible.
Total number of contigs	The total number of contigs assembled.
Number of contigs > 200 bp	The total number of contigs assembled which have a sequence length longer than 200 base pairs.
N50	The N50 is defined as the length of the contig, for which the sum of all contigs of that length or longer equals at least 50 % of the sum of all contigs.
NG50	The NG50 is defined as the length of the contig, for which the sum of all contigs of that length or longer equals at least 50 % of the reference genome size.

6.2. Appendix 2: The quality control parameters included for the evaluation of Nanopore sequences submitted by the participants in EQA 2023.

Parameters	**Description**
Number of (filtered) reads	The number of reads describes the sequence yield, how much was sequenced. The filtered number refers to after filtering to a minimal length of 500bp and average quality score of 12.
Number of (filtered) bases	The total number of base pairs in your reads. The filtered number refers to after filtering to a minimal length of 500bp and average quality score of 12.
Longest read	Length in base pairs of longest read.
Shortest read	Length in base pairs of shortest read.
N50 (filtered) read length	The N50 of all reads after filtering. The N50 is defined as the length of the read, for which the sum of all reads of that length or longer equals at least 50 % of the sum of all base pairs.
Mean/Median (filtered) read length	Mean/median length of all reads, before and after filtering.
Mean/Median (filtered) read quality	Mean/median Q-score of all reads, before and after filtering.
(Filtered) reads >500bp	Number of reads larger than 500bp before and after filtering. Number above thresholds of 1000, 2000, 5000 and 10000bp is likewise stated.
(Filtered) reads quality >10	Number of reads with an average Q-score above 10, before and after filtering. Likewise stated for thresholds of 12 and 20. Note, filtering removes reads of average Q-score <12.
Number of mapped reads	Total number of reads mapped to the reference genome.
Mapped to chromosome/plasmid N	Number of reads mapping to the specific genomic component.
Total assembly size	Total number of base pairs in the assembly.
Number of contigs	The number of produced contigs compared to the number expected in the reference (chromosome + number of plasmids), shown as a fraction.
Number circularized	Number of contigs reported to be circularized by the assembler.
MLST	Identified MLST
Coverage of the reference genome/chromosome/plasmid N (%)	Proportion of the reference genome, chromosome or plasmid (N) covered by reads (this cannot exceed 100%)
Coverage 20/30/40/50x of the reference genome/chromosome/plasmid N (%)	Proportion of the reference genome, chromosome or plasmid N, covered by at least X times of reads. (This cannot exceed 100%).

6.3. Appendix 3: 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 version 2 .9 (2022-05-11)	MLST-2 Database version (2023-06-19)	default	https://cge.food.dtu.dk/services/MLST/
EURGen-RLC-002	web-based pipeline	MLST 2	commercial database	default parameters	https://cge.food.dtu.dk/services/MLST/
EURGen-RLC-003	web-based pipeline	MLST 2 .9 (2022-05-11)	Database version: 2023-06-19	Default	https://cge.food.dtu.dk/services/MLST/
EURGen-RLC-004	local	mlst v2.23	not applicable	default parameters	https://github.com/tseemann/mlst/releases ¤ PubMLST
EURGen-RLC-008	web-based	MLST 2 (CGE webtool)	NA	Select min. depth for an allele - 30x	https://cge.food.dtu.dk/services/MLST/
EURGen-RLC-009	Local	BIFROST	Publicly available database (pubmlst.org)	NA	https://github.com/ssi-dk/bifrost
EURGen-RLC-010	web-based	MLST (v.2 .9)	mlst_db (v. 2023-06-19)	Min. depth for an allele = 5x	https://bitbucket.org/genomicepidemiology/mlst/src/master/ ¤ https://bitbucket.org/genomicepidemiology/mlst_db/src/master/
EURGen-RLC-011	local	srst2	publicly available	default parameters	https://github.com/katholt/srst2
EURGen-RLC-012		pubMLST	NA	NA	NA
EURGen-RLC-014	local	Ridom SeqSphere+ v9 .3	NA	default parameters	NA
EURGen-RLC-015	Web-based pipeline	MLST (Version 2.23)	Publicly available database	NA	NA
EURGen-RLC-016	web based	MLST 2 (CGE) Software version: 2 .9	MLST 2 (CGE) Database version: (2023-06-19)	default	https://cge.food.dtu.dk/services/MLST/
EURGen-RLC-017	NA	BioNumerics 8.1	NA	NA	NA
EURGen-RLC-018	web-based pipeline	MLST 2 , Software version: 2 .9 (2022-05-11)	PubMLST.org. Database version: (2023-06-19) ¤	default parameters	https://cge.food.dtu.dk/services/MLST/

Laboratory	Pipeline type	Software	Database	Parameters of the software	URL of the software or database
EURGen-RLC-019	7gene MLST, rMLST, cgMLST. Gathered and locally hosted schemas from pubmlst, enterobase and ridom.	1. mlst, version 2.19 , 2. chewbbaca, 3.1.2, (scheme from https://cgmlst.org/ncs. 3. PubMLST RESTful API for rMLST	publicly available databases. ¤ 1. mlst, version 2.19 , https://github.com/tseemann/mlst ¤ 2. chewbbaca, version 3.1.2, https://github.com/B-UMMI/chewBBACA (scheme from https://cgmlst.org/ncs. Scheme versions - Escherichia coli : 2023-05-17; Klebsiella : 2023-05-05) ¤ 3. PubMLST RESTful API for rMLST, https://pubmlst.org/species-id/species-identification-via-api	default parameters	1. mlst, version 2.19 , https://github.com/tseemann/mlst ¤ 2. chewbbaca, version 3.1.2, https://github.com/B-UMMI/chewBBACA (scheme from https://cgmlst.org/ncs. Scheme versions - Escherichia coli : 2023-05-17; Klebsiella : 2023-05-05) ¤ 3. PubMLST RESTful API for rMLST, https://pubmlst.org/species-id/species-identification-via-api
EURGen-RLC-020	Ridom SeqSphere+, version 9	NA	NA	Default	https://www.ridom.de/seqsphere/
EURGen-RLC-021	NA	Seqsphere, version 9 .2 (2023-04)	NA	default	NA
EURGen-RLC-022	web-based pipeline	CGE:MLST Software 2 .9 (2022-05-11)	CGE:MLST Database (2023-06-19)	default	NA
EURGen-RLC-023	local	mlst v.2.23	publicly available mlst v.2.23	default	https://github.com/tseemann/mlst
EURGen-RLC-024	web-based pipeline	publicly available software: Center for Genomic Epidemiology (MLST v. 2), Institut Pasteur MLST,	publicly available database Center for Genomic Epidemiology (MLST v. 2), Institut Pasteur MLST,	default parameters	NA
EURGen-RLC-026	Web-based	Publicly available software	Publicly available database	Default parameters	https://cge.food.dtu.dk/services/MLST/
EURGen-RLC-027	Center for Genomic Epidemiology	SeqSphere+	NA	Default	https://www.genomicepidemiology.org/
EURGen-RLC-028	Web-based pipeline	MLST 2 .9	Database version 2023-06-19	Select min. depth for an allele 5x, for Acinetobacter baumanni scheme #2 (Pasteur scheme)	https://cge.food.dtu.dk/services/MLST/
EURGen-RLC-029	local	SeqSphere+ 9 .2 (Ridom)	PubMLST via SeqSphere+ (Ridom)	default	https://www.ridom.de/seqsphere/

Laboratory	Pipeline type	Software	Database	Parameters of the software	URL of the software or database
EURGen-RLC-030	Local pipeline	mlst, 2.22.1	PubMLST, BIGSdb Version 1.42.3	Default parameters	https://github.com/tseemann/mlst ¤ https://pubmlst.org/
EURGen-RLC-031	Web-based pipeline: MLST CGE	NA	NA	Default parameters	https://cge.food.dtu.dk/services/MLST/
EURGen-RLC-032	NA	NA	NA	NA	NA
EURGen-RLC-033	NA	Center for Genomic Epidemilology MLST 2	2023-06-19	Default	https://cge.food.dtu.dk/services/MLST/
EURGen-RLC-034	local	commercial software; SeqSphere+ 9 .5	Publicly available database; A. baumannii and P. aeruginosa via PubMLST (A. baumannii Oxford scheme), E.coli via Enterobase (Warwick scheme) and Klebsiella pneumoniae via Institute Pasteur (Pasteur scheme)	default parameters	https://pubmlst.org/, https://enterobase.warwick.ac.uk/, https://bigsdb.pasteur.fr/
EURGen-RLC-035	Web-based	NA	MLST 2 - (Software version: 2 .9 (2022-05-11),Database version: (2023-06-19) ¤ cgMLSTFinder 1.2 - Software version: 1 .1 (2021-08-29)	NA	https://cge.food.dtu.dk/services/MLST/ ¤ https://cge.food.dtu.dk/services/cgMLSTFinder/

NA; Not Applicable

6.4. Appendix 4: 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 2.1 version 2 .1 (2020-07-01); Mobile Element Finder version v1 .3 (2020-10-09)	PlasmidFinder: Database version (2023-01-18), MGE: Database version v1 .2 (2020-06-09)	default	https://cge.food.dtu.dk/services/PlasmidFinder/ ; https://cge.food.dtu.dk/services/MobileElementFinder/
EURGen-RLC-002	web-based pipeline	PlasmidFinder 2.1	commercial database	default parameters	https://cge.food.dtu.dk/services/PlasmidFinder/
EURGen-RLC-003	web-based pipeline	PlasmidFinder 2 .1	Enterobacteriales Database version: 2023-01-18	Minimum % identity: 95% ¤ Minimum % coverage: 80%	https://cge.food.dtu.dk/services/PlasmidFinder/
EURGen-RLC-004	locally installed tools	PlasmidFinder; ABRicate v1	PlasmidFinder DB v2023-01-18;	default parameters	https://bitbucket.org/genomicepidemiology/plasmidfinder/src/master/; https://github.com/tseemann/abricate
EURGen-RLC-008		PlasmidFinder 2.1	NA	default parameters	https://cge.food.dtu.dk/services/PlasmidFinder/
EURGen-RLC-009	Local	BIFROST	PlasmidFinder	Minimum length 90% and minimum identity 80%	https://bitbucket.org/genomicepidemiology/plasmidfinder_db/src/master/ (https://github.com/ssi-dk/bifrost)
EURGen-RLC-010	NA	PlasmidFinder (v.2 .1)	plasmidfinder_db (v. 2023-01-18)	Threshold for minimum identity 95% ¤ Minimum coverage 60%	https://bitbucket.org/genomicepidemiology/plasmidfinder/src/master/ ¤ https://bitbucket.org/genomicepidemiology/plasmidfinder_db/src/master/
EURGen-RLC-011	web-based	CGE plasmid finder	CGE:n plasmid database	default	NA
EURGen-RLC-012	Local	From plasmidfinder and added specific data from Acinetobacter and Pseudomonas	plasmidfinder	NA	NA
EURGen-RLC-014	local and web-based	Ridom SeqSphere+ v9 .3, PlasmidFinder 2.1	NA	default parameters: minimum length 60% and minimum identity 95%	https://cge.food.dtu.dk/services/PlasmidFinder/
EURGen-RLC-015	Web-based pipeline	PlasmidFinder	NA	NA	https://cge.food.dtu.dk/services/PlasmidFinder/
EURGen-RLC-016	web-based	PlasmidFinder 2.1 (CGE) Software version: 2 .1	PlasmidFinder 2.1 (CGE) Database version: (2023-01-18)	default	https://cge.food.dtu.dk/services/PlasmidFinder/

Laboratory	Pipeline type	Software	Database	Parameters of the software	URL of the software or database
EURGen-RLC-017	NA	Bionumerics 8.1, E.coliplug in	NA	NA	NA
EURGen-RLC-018	web-based pipeline	PlasmidFinder 2.1, Software version: 2 .1 (2020-07-01)	Database version: (2023-01-18), Test sequence	default parameters	https://cge.food.dtu.dk/services/PlasmidFinder/
EURGen-RLC-019	local	publicly available software ¤ 1. plasmidfinder, version 2023-03-17, https://bitbucket.org/genomicepidemiology/plasmidfinder/src/master/, database version 2023-03-17	publicly available database ¤ 1. plasmidfinder, version 2023-03-17, https://bitbucket.org/genomicepidemiology/plasmidfinder/src/master/, database version 2023-03-17	default	1. plasmidfinder, version 2023-03-17, https://bitbucket.org/genomicepidemiology/plasmidfinder/src/master/, database version 2023-03-17
EURGen-RLC-020		Ridom SeqSphere+, version 9	NA	Default	https://www.ridom.de/seqsphere/
EURGen-RLC-021	NA	NA	PlasmidFinder-2	default	https://cge.food.dtu.dk/services/PlasmidFinder-2 /
EURGen-RLC-022	web-based pipeline	CGE-Plasmid Finder Software 2 .1 (2020-07-01)	CGE-Plasmid Finder Database (2023-01-18)	default	NA
EURGen-RLC-023	local	Plasmidfinder v.2.1.6	Publicly available Plasmidfinder v.2.1.6	minimum coverage 90% and minimum identity 90%	https://bitbucket.org/genomicepidemiology/plasmidfinder/src/master/
EURGen-RLC-024	web-based pipeline ¤ tested only for E. coli and K. pneumoniae	MobileElementFinder Software version: v1 .3 (2020-10-09), ¤ PlasmidFinder 2.1, Software version: 2 .1 (2020-07-01)	MobileElementFinder Database version: v1 .2 (2020-06-09); ¤ PlasmidFinder 2.1, Database version: (2023-01-18)	default parameters	NA
EURGen-RLC-026	Web-based	Publicly available software	Publicly available database	Default parameters	https://cge.food.dtu.dk/services/PlasmidFinder/
EURGen-RLC-027	PlasmidFinder 2.1	NA	NA	minimum length 60% and minimum identity 90%	https://cge.food.dtu.dk/services/PlasmidFinder/
EURGen-RLC-028	Web-based pipeline	PlasmidFinder 2 .1	Database version 2023-01-18	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	PlasmidFinder 2.1 Database version: (2023-01-18)	dafault	https://cge.food.dtu.dk/services/PlasmidFinder/
EURGen-RLC-030	Local pipeline	PlasmidFinder, 2 .1, abricate, 1 .1	plasmidfinder_db, version 2023-01-18	Default parameters	https://bitbucket.org/genomicepidemiology/plasmidfinder_db/src/master/ ¤ https://github.com/tseemann/abricate
EURGen-RLC-031	Web-based pipeline:	To screen plasmids for Aba (Eurgen2023-01) we used	NA	Default parameters	https://cge.food.dtu.dk/services/PlasmidFinder/;

Laboratory	Pipeline type	Software	Database	Parameters of the software	URL of the software or database
	PlasmidFinder CGE (WS1: K. pneumoniae and E. coli). Local pipeline for WS2-Aba.	the AcinetobacterPlasmidTyping			https://github.com/MehradHamidian/AcinetobacterPlasmidTyping;
EURGen-RLC-032	NA	NA	NA	NA	NA
EURGen-RLC-033	Center for Genomic Epidemilology	PlasmidFinder 2.1	2023-01-18	Default	https://cge.food.dtu.dk/services/PlasmidFinder/
EURGen-RLC-034	local	publicly available software	PlasmidFinder	minimum length 60% and minimum identity 95%	https://bitbucket.org/genomicepidemiology/workspace/projects/DB
EURGen-RLC-035	NA	PlasmidFinder 2.1 - Software version: 2 .1 (2020-07-01) ¤ MobileElemetFinder - Software version: v1 .3 (2020-10-09) ¤	PasmidFinder Database version: (2023-01-18) ¤ MobileElementFinder Database version: v1 .2 (2020-06-09)	NA	https://cge.food.dtu.dk/services/PlasmidFinder/ ¤ https://cge.food.dtu.dk/services/MobileElementFinder/

NA; Not Applicable

6.5. Appendix 5: 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	web-based	ResFinder software: version (2022-08-08) ¤ AMRFinderPlus software: version 3.11.2	ResFinder database: version (2022-05-24) ¤ AMRFinderPlus database: version 2022-12-19.1	default	https://cge.food.dtu.dk/services/ResFinder/
EURGen-RLC-002	web-based pipeline	ResFinder 4.1	commercial database	default parameters	https://cge.food.dtu.dk/services/ResFinder/
EURGen-RLC-003	web-based pipeline	ResFinder 4.1 and PointFinder software: (2022-08-08)	ResFinder database: (2022-05-24) ¤ PointFinder database: (2022-04-22)	Minimum length 60% and minimum identity 90%	https://cge.food.dtu.dk/services/ResFinder/
EURGen-RLC-004	locally installed tools	AMRFinderPlus v3.11.17; ResFinder v4.3.1; RGI v6 .2	AMRFinderPlus DB v2023-07-13.2; ResFinder DB v2022-05-24; RGI DB v3.2.6	default parameters	NA
EURGen-RLC-008		ResFinder 4.1	NA	default parameters	https://cge.food.dtu.dk/services/ResFinder/
EURGen-RLC-009	local	BIFROST	ResFinder 4.1	Minimum length 90% and minimum identity 90%,	https://bitbucket.org/genomicepidemiology/resfinder_db/src/master/
EURGen-RLC-010	NA	ResFinder (v.4.1)	resfinder_db (v. 2022-05-24) ¤	Threshold for ID 90%, minimum length 60%	https://bitbucket.org/genomicepidemiology/resfinder/src/master/ ¤ https://bitbucket.org/genomicepidemiology/resfinder_db/src/master/ ¤
EURGen-RLC-011	web-based and local	srst2 and CGE ResFinder	CARD version 3 .8 and ARG-ANNOT	default	https://github.com/katholt/srst2
EURGen-RLC-012	Local	detector 1	database ARM(102) (generated from resfinder, CARD and NCBI)	minimum length 80% and minimum identity 70%	NA
EURGen-RLC-014	local and web-based	NCBI AMRFinderPlus implemented in Ridom SeqSphere+ v9 .3 ¤ ResFinder 4.1	NA	ResFinder 4.1 with default parameters (minimum length 60% and minimum identity 90%)	ResFinder 4.1: https://cge.food.dtu.dk/services/ResFinder/
EURGen-RLC-015	Web-based pipeline	ResFinder 4.3.3	NA	NA	http://genepi.food.dtu.dk/resfinder

Laboratory	Pipeline type	Software	Database	Parameters of the software	URL of the software or database
EURGen-RLC-016	web-based	ResFinder-4.1; RGI 6 .2, CARD 3.2.7	ResFinder-4.1 ResFinder database: (2022-05-24); ¤ RGI 6 .2, CARD 3.2.7	default	https://card.mcmaster.ca/analyze/rgi, https://cge.food.dtu.dk/services/ResFinder/
EURGen-RLC-017	NA	NA	BioNumerics 8.1, E.coli plug in	Both 99%	NA
EURGen-RLC-018	web-based pipeline	ResFinder 4.1, ResFinder and PointFinder software: (2022-08-08)	ResFinder database: (2022-05-24) ¤ PointFinder database: (2022-04-22) ¤	default parameters	https://cge.food.dtu.dk/services/ResFinder/
EURGen-RLC-019	local	publicly available software ¤ 1. rgi, version 5.2.1 card_v3.1.4 ¤ 2. amrfinder+, version 3.10.42 ¤ 3. resfinder, version 4.1.11	publicly available database ¤ 1. rgi, version 5.2.1, card_v3.1.4 ¤ 2. amrfinder+, version 3.10.42 database version 2022-10-11.2 ¤ 3. resfinder, version 4.1.11, database version 2023-03-29	default	https://github.com/arpcard/rgion 3.10.42, https://github.com/ncbi/amr https://bitbucket.org/genomicepidemiology/resfinder/src/master/,
EURGen-RLC-020	NA	Ridom SeqSphere+, version 9	NA	Default	https://www.ridom.de/seqsphere/
EURGen-RLC-021	NA	Seqsphere, version 9 .2 (2023-04)	AMRFinderPlus database version: 2022-12-19.1 ; ResFinder-4.1 ; CARD 3.2.7	default	https://cge.food.dtu.dk/services/ResFinder/ ; https://card.mcmaster.ca/analyze/rgi
EURGen-RLC-022	web-based pipeline	CGE-Resfinder 4.1 (2022-08-08)	CGE-ResFinder (2022-05-24),	default	NA
EURGen-RLC-023	local	AMRFinderPlus v.3.11.2, Kleborate v.2.2	Publicly available AMRFinderPlus v.2023-04-17.1, Kleborate v.2.2	minimum coverage 90% and minimum identity 90%, secondarily minimum coverage 60% and minimum identity 60%	https://github.com/ncbi/amr, https://github.com/klebgenomics/Kleborate/wiki
EURGen-RLC-024	web-based pipeline	publicly available software: ¤ ResFinder 4.1 and PointFinder software: (2022-08-08)	ResFinder database: (2022-05-24) ¤ PointFinder database: (2022-04-22)	default parameters	NA
EURGen-RLC-026	Web-based pipeline	Publicly available software	Publicly available database	Default parameters	https://cge.food.dtu.dk/services/ResFinder/
EURGen-RLC-027	ResFinder 4.1 ¤ https://cge.food.dtu.dk/services/ResFinder/	AMRFinderPlus through SeqSphere+	NA	default	NA

Laboratory	Pipeline type	Software	Database	Parameters of the software	URL of the software or database
EURGen-RLC-028	Web-based pipeline	ResFinder 4.1	ResFinder database 2022-05-24	Select threshold for %ID: 90%; Select minimum length: 60%	https://cge.food.dtu.dk/services/ResFinder/
EURGen-RLC-029	local	SeqSphere+ 9 .2 (Ridom)	NCBI AMRFinderPlus 1.1 via SeqSphere+	dafault	https://www.ridom.de/seqsphere/
EURGen-RLC-030	Local pipeline	AMRfinderPlus, ResFinder	Resfinder_db, version 2.1.1, 2023-03-03 ¤ AMRFinderPlus database, version 2023-07-13 ¤	Default parameters	https://bitbucket.org/genomicepidemiology/resfinder_db, https://ftp.ncbi.nlm.nih.gov/pathogen/Antimicrobial_resistance/AMRFinderPlus/database/latest/ ¤ https://bitbucket.org/genomicepidemiology/resfinder/src/master/ ¤ https://github.com/ncbi/amr
EURGen-RLC-031	Local and web-based pipeline	ARIBA v. 2.6.2 (local); ResFinder v. 4.1 from CGE (web-based)	ARIBA- CARD db	Default parameters for ARIBA; minimum length 60% and minimum identity 98% for ResFinder	https://github.com/sanger-pathogens/ariba; https://cge.food.dtu.dk/services/ResFinder/
EURGen-RLC-032	NA	NA	NA	NA	NA
EURGen-RLC-033	Center for Genomic Epidemiology	ResFinder 4.1/AMRFinder	2022-08-08	mininum length 60% and minimum identity 80%	https://cge.food.dtu.dk/services/ResFinder/
EURGen-RLC-034	local	publicly available software	Resfinder	minimum length 60% and minimum identity 95%	https://bitbucket.org/genomicepidemiology/workspace/projects/DB
EURGen-RLC-035	NA	ResFinder 4.1, RGI web portal 6.0.2	ResFinder database: RGI CARD 3.2.7	Default parameters	NA

NA; Not Applicable

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