DTU Genomic Proficiency Test 2022

A Guide on the Interpretation of the Submitted Data and the Scoring System

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1. FOREWORD

This document is provided to the EURL-AR and SEQAFRICA networks, participants of the DTU Genomic proficiency test (PT) 2022, as a guide to assist in performance evaluation. In the following sections, a short introduction and overviews of the submitted data (anonymized) are presented for each AMR category, i.e., chromosomal mutations, genes and gene variants, as well as the predicted AMR phenotype. Moreover, the multi-locus sequence typing (MLST) data are discussed. Finally, the scoring system and overviews of the obtained scores are presented in detail. Please note that participants' evaluation is not expressed on a pass/fail basis; therefore, the participants are encouraged to self-evaluate their performance, applying internal acceptance criteria.

2. INTRODUCTION

The participants of DTU Genomic PT 2022 were requested to perform whole genome sequencing (WGS) and bioinformatics analysis on two strains of each of the following organisms: *Staphylococcus aureus* (n=2), *Escherichia coli* (n=2) and *Enterococcus faecalis/E. faecium* (n=2). The codes used for each strain in Genomic PT 2022 are presented in Table 1. For each strain, two types of DNA samples were analysed: 1) DNA isolated from live cultures, referred to as "BACT", and 2) pre-prepared DNA, referred to as "DNA". The downstream processing of the samples is presented in Figure 1.

Table 1. Strain*s included in DTU Genomic PT 2022. Two types of samples were analysed for each* strain*: DNA isolated from live cultures (BACT) and pre*-prepared *DNA (DNA).*

Organism	Strain code	Type of sample
Staphylococcus aureus	GENOMIC22-001	BACT/DNA
	GENOMIC22-002	BACT/DNA
Escherichia coli	GENOMIC22-003	BACT/DNA
	GENOMIC22-004	BACT/DNA
Enterococcus faecium	GENOMIC22-005	BACT/DNA
E. faecalis	GENOMIC22-006	BACT/DNA

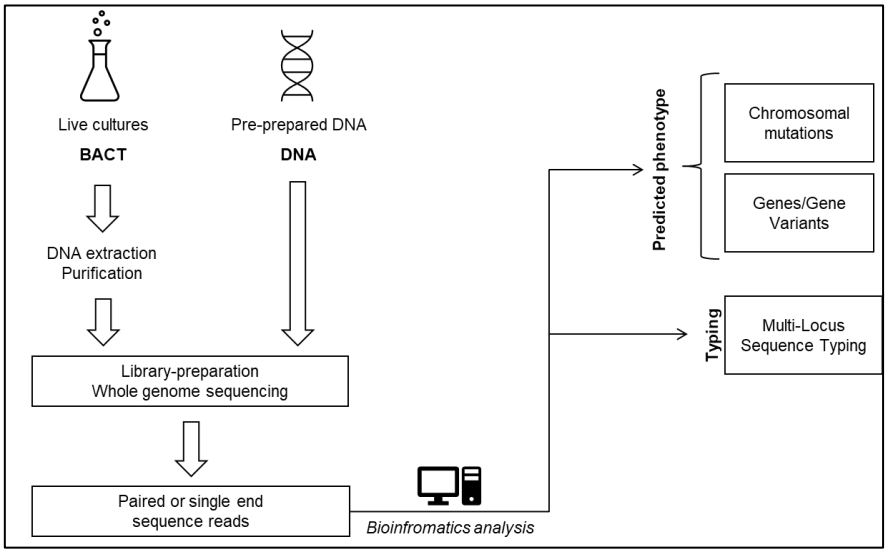

Figure 1. Schematic overview of the downstream processing of live culture samples (BACT) and pre-prepared *DNA samples (DNA).*

The participants were requested to report data on the prediction of AMR determinants towards a limited number of antimicrobials for each species (see Table 2), including chromosomal mutations and genes or gene variants. Based on the identified genotype, the participants had to submit the potential predicted AMR phenotypic profile for each strain. The platform used for data submission (DTU webtool) assigned scores to each submitted result, i.e., chromosomal mutation, gene or gene variant and predicted AMR profile. A positive score (score=1) was assigned for each submitted expected result, while a zero score (score=0) was assigned for cases where expected results were not reported, or when unexpected results were reported. The participants can see their score for each submitted result in their individual evaluation report, which can be downloaded from the webtool.

Table 2. Overview of antimicrobials included in DTU Genomic PT 2022, for S. aureus, E. coli and E. faecalis/E. faecium.

CLASS	ANTIMICROBIAL	ABBREVIATION	*S. aureus*	*E. coli*	*E. faecalis/ E. faecium*
Aminoglycosides	Amikacin	AMI	-	x	-
	Gentamicin	GEN	x	x	x
	Kanamycin	KAN	x	-	-
	Streptomycin	*STR*	x	-	-
Amphenicols	Chloramphenicol	CHL	x	x	x
Beta-lactams	Ampicillin	AMP	-	x	x
	Cefepime	FEP	-	x	-
	Cefotaxime	FOT	-	x	-
	Cefoxitin	FOX	x	x	-
	Ceftazidime	TAZ	-	x	-
	Ertapenem	ETP	-	x	-
	Imipenem	IMI	-	x	-
	Meropenem	MERO	-	x	-
	Penicillin	PEN	x	-	-
	Temocillin	TRM	-	x	-
Folate pathway antagonists	Sulfamethoxazole	SMX	x	x	-
	Trimethoprim	TMP	x	x	-
Glycopeptides	Teicoplanin	TEI	-	-	x
	Vancomycin	VAN	x	-	x
Lincosamides	Clindamycin	CLI	x	-	-
Macrolides	Azithromycin	AZI	-	x	-
	Erythromycin	ERY	x	-	x
Oxazolidinones	Linezolid	LZD	x	-	x
Pleuromutilins	Tiamulin	TIA	x	-	-
Polymyxins	Colistin	COL	-	x	-
Pseudomonic acids	Mupirocin	MUP	x	-	-
Quinolones	Ciprofloxacin	CIP	x	x	x
	Nalidixic acid	NAL	-	x	-
Rifamycins	Rifampin	RIF	x	-	-
Steroid antibacterials	Fusidate	FUS	x	-	-
Tetracyclines	Tetracycline	TET	x	x	x
	Tigecycline	TGC	-	x	x

3. PARTICIPATION

Twenty-six (n=26) laboratories, from the EURL-AR and SEQAFRICA networks, participated in the Genomic PT 2022, and were assigned codes starting from 2022-01 to 2022-26. One laboratory (2022-20) is excluded from the present document, because of a delay in submitting data. Participants could sing-up for the analysis of each sample individually. The level of participation of each laboratory in the analysis of the

different samples included in Genomic PT 2022 is presented in Table 3. Two laboratories analysed the *E. coli* samples only, and four laboratories analysed only the *S. aureus* and *E. coli* samples. Additionally, one laboratory submitted data solely for the BACT samples across all strains. The remaining participants analyzed all twelve samples. Overall, 262 sets of data were submitted for evaluation.

Table 3. Summary of participants sample analyses in the DTU Genomic Proficiency Test 2022.

LAB ID	*S. aureus*				*E. coli*				*E. faecalis/E. faecium*				SUM
	GENOMIC22-001		GENOMIC22-002		GENOMIC22-003		GENOMIC22-004		GENOMIC22-005		GENOMIC22-006		
	BACT	DNA	BACT	DNA	BACT	DNA	BACT	DNA	BACT	DNA	BACT	DNA	
2022-01	x	x	x	x	x	x	x	x	x	x	x	x	12
2022-02	x	-	x	-	x	-	x	-	x	-	x	-	6
2022-03	x	x	x	x	x	x	x	x	x	x	x	x	12
2022-04	-	-	-	-	x	x	x	x	-	-	-	-	4
2022-05	x	x	x	x	x	x	x	x	x	x	x	x	12
2022-06	x	x	x	x	x	x	x	x	x	x	x	x	12
2022-07	x	x	x	x	x	x	x	x	x	x	x	x	12
2022-08	-	-	-	-	x	x	x	x	-	-	-	-	4
2022-09	x	x	x	x	x	x	x	x	x	x	x	x	12
2022-10	x	x	x	x	x	x	x	x	x	x	x	x	12
2022-11	x	x	x	x	x	x	x	x	-	-	-	-	8
2022-12	x	x	x	x	x	x	x	x	-	-	-	-	8
2022-13	x	x	x	x	x	x	x	x	x	x	x	x	12
2022-14	x	x	x	x	x	x	x	x	x	x	x	x	12
2022-15	x	x	x	x	x	x	x	x	x	x	x	x	12
2022-16	x	x	x	x	x	x	x	x	x	x	x	x	12
2022-17	x	x	x	x	x	x	x	x	x	x	x	x	12
2022-18	x	x	x	x	x	x	x	x	x	x	x	x	12
2022-19	x	x	x	x	x	x	x	x	x	x	x	x	12
2022-21	x	x	x	x	x	x	x	x	x	x	x	x	12
2022-22	x	x	x	x	x	x	x	x	x	x	x	x	12
2022-23	x	x	x	x	x	x	x	x	x	x	x	x	12
2022-24	x	x	x	x	x	x	x	x	-	-	-	-	8
2022-25	x	x	x	x	x	x	x	x	-	-	-	-	8
2022-26	x	x	x	x	x	x	x	x	x	x	x	x	12
SUM	**23**	**22**	**23**	**22**	**25**	**24**	**25**	**24**	**19**	**18**	**19**	**18**	**262**

4. EXPECTED RESULTS

To determine the expected AMR results for each strain, i.e. the AMR chromosomal mutations and genes as well as the predicted AMR phenotype, the EURL-AR used Resfinder v4.1, database version 2022-10-26 and Pointfinder, database version 2022-11-25. Moreover, the sequences were run in the latest versions of CARD and AMRFinderPlus for comparison. Closed genomes for all strains were used to generate the expected data. Sequence type was determined with MLST v2.0, database v2022-08-01. All tools were run with default parameters.

5. SCORING SYSTEM

There is no pass or fail evaluation and the scoring principle in the individual evaluation reports of the participating laboratories is as follows:

- Score=1 for submitting expected results
- Score=0 for not submitting expected results
- No score (blank) for special cases, each of which is explained individually, in the following sections of the present document.

The predicted AMR phenotype results were evaluated with an "all-or-none" approach, i.e., were positively scored (score=1) when the exact and complete expected phenotypic AMR profile was reported; otherwise, submitted profiles obtained a zero score (score=0). In the present report, in order to evaluate the performance of the participants in more detail, individual scores were assigned to each AMR phenotype submitted, instead of scoring the entire phenotypic AMR profile.

6. DATA ANALYSIS – AMR

6.1. *S. aureus*, GENOMIC22-001

The AMR genes and the potential predicted AMR phenotypes identified by the EURL-AR pipeline for strain GENOMIC22-001 are presented in Table 4. Moreover, a schematic presentation of the location of the idenitified AMR genes is presented in Figure 2. The EURL-AR pipeline identified perfect hits (100,00% identity and 100,0% coverage of the reference gene) for eight genes: *str*, *fexA*, *blaZ*, *dfrG*, *lnu(B)*, *cfr*, *tet(M)* and *tet(K)*. Not submitting any of these genes, and/or the respective potential predicted AMR phenotype, was scored with a zero. There were controversies regarding three hits:

1) The *mecA* gene (hit in database: NC_007168) was identified in the sequence with 99,95% identity and 100,0% coverage. There is a single nucleotide substitution at position 682 of the reference gene (codon GCA becomes ACA in the genome), expected to lead to an amino acid substitution at position 228 of the protein (reference: WP_063852638) from A to T. This is a transition from a hydrophobic to a polar, non-charged amino acid. Based on the protein structure of the penicillin-binding protein 2a (PBP2a) from a methicillin-resistant *S. aureus*, provided in Lim and Strynadka, 2022[1], this amino acid is expected to be in the non-penicillin binding domain of the protein, therefore not at the transpeptidase domain. Based on this, it is expected that the protein is functional in this strain and that it confers resistance to FOX, despite the single amino acid substitution. Therefore, not submitting the *mecA* gene received a zero score.

2) The *lsa(B)* gene (hit in database: AJ579365) was identified in the sequence with 99,80% identity and 100,0% coverage. There are three base substitutions at positions 496, 497 and 535 of the reference (codon AAT changes to TTT and codon AGT changes to TGT). These mutations are expected to lead to two amino acid substitutions, from N to F, and from S to C, which have very different chemical properties and could have an impact on the protein structure and functionality. A solved structure for the coding protein of *lsa(B)* gene was not available and therefore this cannot be investigated further. Moreover, the strain is phenotypically resistant to CLI, however this can be attributed to other genes too identified in the sequence. Because of the above, it was decided to blank the scores for not reporting *lsa(B)* gene, i.e., score=1 if reported, score=blank if not reported.

3) The *lsa(E)* gene (hit in database: JX560992) was identified in the sequence with 99,93% identity and 100,0% coverage. There is one base substitution at position 595 of the reference (codon ACT changes to CCT) expected to lead to a single amino acid substitution from T to P at position

[1] Lim, D., Strynadka, N. Structural basis for the ß lactam resistance of PBP2a from methicillin-resistant *Staphylococcus aureus*. *Nat Struct Mol Biol* **9**, 870–876 (2002). https://doi.org/10.1038/nsb858

199 of the protein (reference AFU35065.1). A protein structure was not available to further investigate the potential impact of the above change to a protein level, and it was therefore decided to blank the scores for not reporting *lsa(E)* gene, i.e., score=1 if reported, score=blank if not reported.

Table 4. AMR genes identified by the EURL-AR pipeline for strain *GENOMIC22-001 and the respective predicted AMR phenotypes. Details regarding the hits in the ResFinder database as well as the percent identity (%ID) and coverage (%COV) to the reference gene are presented (hits from closed genomes).*

Antimicrobial class	AMR Gene	Predicted AMR Phenotype (relevant for Gen. PT 2022)	Hit in database (Accession number)	Location	Position	%ID	%COV	Score if gene or predicted phenotype not reported
Aminoglycoside	*str*	STR	FN435330	plasmid_2	3258..4106	100.00	100.0	0
Amphenicol	*fexA*	CHL	AJ549214	plasmid_1	21873..23300	100.00	100.0	0
Beta-lactam	*blaZ*	PEN	JBTH01000015	chromosome	1743685..1744533	100.00	100.0	0
	mecA	FOX	NC_007168	chromosome	52811..54817	99.95	100.0	0
Folate pathway antagonist	*dfrG*	TMP	AB205645	chromosome	1740446..1740943	100.00	100.0	0
Lincosamide	*lnu(B)*	CLI	JQ861959	chromosome	1191576..1192379	100.00	100.0	0
Lincosamide, Streptogramin A	*lsa(B)*	CLI	AJ579365	plasmid_1	29775..31253	99.80	100.0	**Blank**
Lincosamide, Streptogramin A, Pleuromutilin	*lsa(E)*	CLI, TIA	JX560992	chromosome	1190038..1191522	99.93	100.0	**Blank**
Oxazolidinone, Amphenicol, Lincosamide, Streptogramin A, Pleuromutilin	*cfr*	CHL, CLI, LZD, TIA	AM408573	plasmid_1	27221..28270	100.00	100.0	0
Tetracycline	*tet(K)*	TET	U38656	chromosome	39448..40827	100.00	100.0	0
	tet(M)	TET	AM990992	chromosome	1954153..1956072	100.00	100.0	0

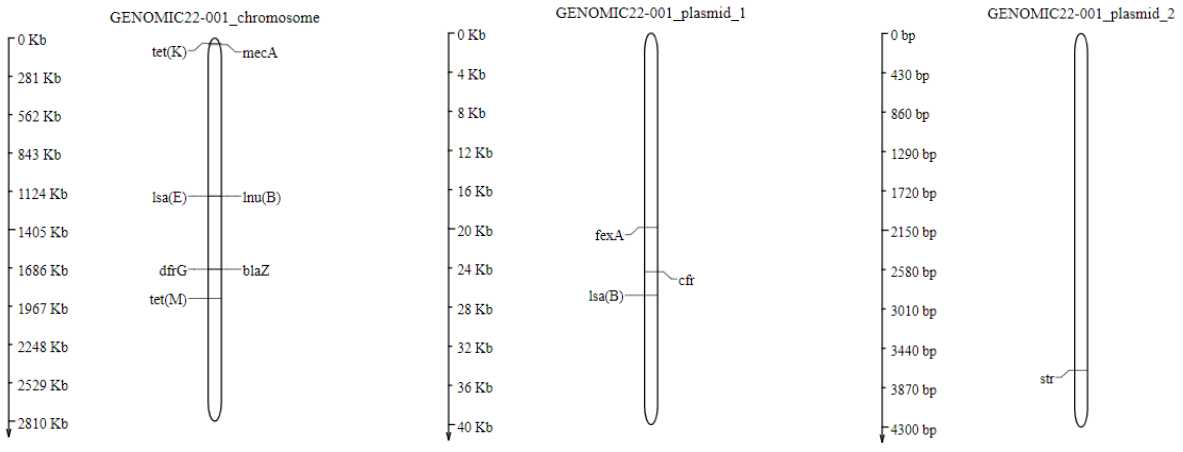

Figure 2. Schematic presentation of the location of the AMR genes identified by the EURL-AR pipeline for S. aureus strain GENOMIC22-001. The figure was generated using the online genetic map drawing tool MG2C (http://mg2c.iask.in/mg2c_v2.0/).

From the 25 participating laboratories, 23 analysed GENOMIC22-001-BACT and 22 GENOMIC22-001-DNA (see Table 3). An overview of the submitted AMR genes and the predicted AMR phenotype is

presented in Figure 3, Figure 4 and Figure 5. The majority of participants identified and reported the expected AMR genes and the respective predicted AMR phenotype (individual antimicrobials); however, only 11 participants submitted the expected predicted AMR profile. For the participants who did not report the expected genes, EURL-AR identified them in the submitted DNA sequences of these participants, using ResFinder. A few unexpected genes were reported:

1) *ant(9)-Ia*: the EURL-AR pipeline identified a hit for this gene too (hit in database: X02588) with 94.24% identity and 99.5% coverage. The hit is not perfect; therefore, the functionality of the gene is questionable. Regardless of the above, *ant(9)-Ia* is predicted to confer resistance to spectinomycin, which is not included in the list of the relevant antimicrobials for *S. aureus* (Table 2) for GENOMIC PT 2022, so it should have not been reported. Reporting of this gene received a score ´0´.

2) *Cfr(D)*, *dfrB1*, *aph(3")-Ib* and *blaL*: the EURL-AR pipeline did not identify these genes in the submitted sequences of the participants. Reporting of these genes received a score ´0´.

3) *erm(C)*: this gene was reported by three laboratories: 2022-14 (DNA), 2022-15 (BACT and DNA) and 2022-16 (BACT). The EURL-AR pipeline identified a perfect hit for *erm(C)* gene in the raw reads of the DNA sample of laboratory 2022-14. For the other samples, the hits were not perfect. This was an unexpected finding, as there was no hit identified for *erm(C)* either at the closed or assembled genome or the raw reads of this strain, by the EURL-AR pipeline. We do not know why hits for *erm(C)* were identified in the sequences of the laboratories above. The *erm(C)* gene is predicted to confer resistance to ERY; the strain is however phenotypically susceptible to ERY. Because of the above, reporting of *erm(C)* was handled as a mistake, and received a score ´0´.

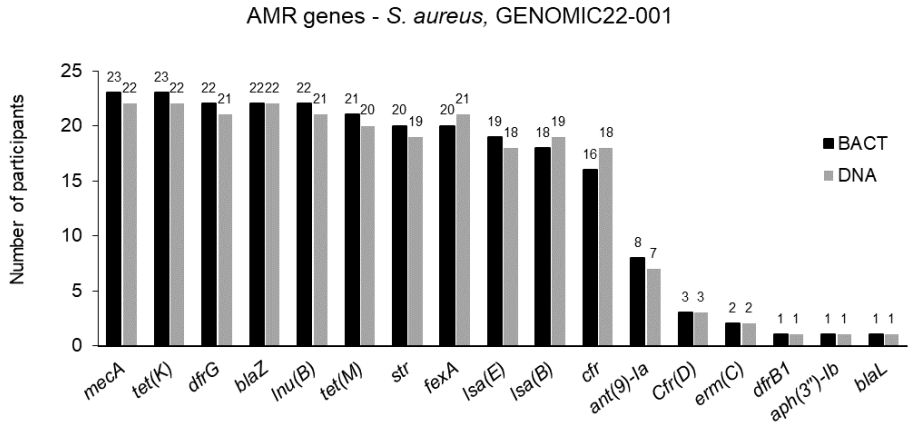

Figure 3. Overview of submitted AMR genes for S. aureus strain GENOMIC22-001.

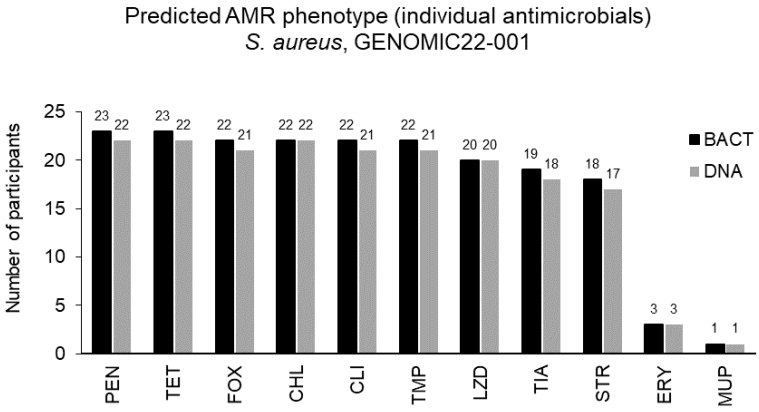

Figure 4. Overview of submitted predicted AMR phenotypes (individual antimicrobials) for S. aureus strain GENOMIC22-001.

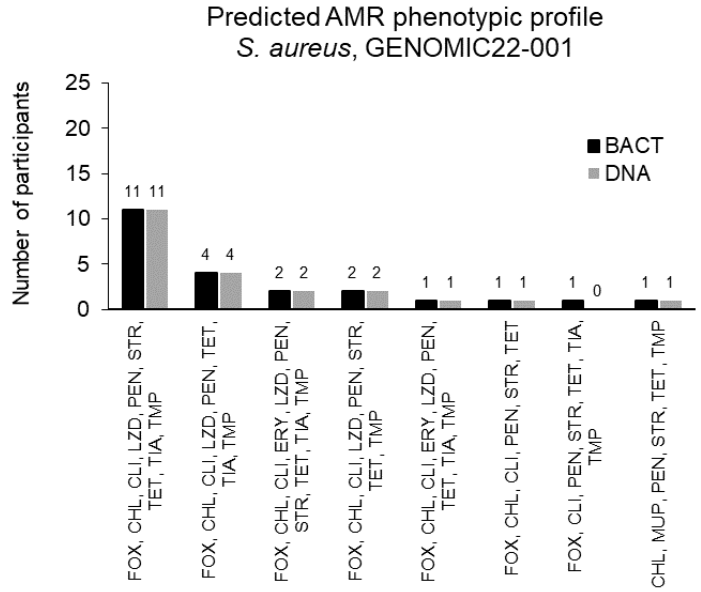

Figure 5. Overview of submitted predicted AMR profiles for S. aureus strain GENOMIC22-001.

6.2. *S. aureus*, GENOMIC22-002

The AMR chromosomal mutations and genes as well as the predicted AMR phenotypes identified by the EURL-AR pipeline for strain GENOMIC22-002 are presented in Table 5 and Table 6. Moreover, a schematic presentation of the location of the idenitified AMR genes is presented in Figure 6. The EURL-AR pipeline, identified two chromosomal mutations (*grlA*_S80Y and *gyrA*_S84L) as well as perfect hits (100,00% identity and 100,0% coverage of the reference gene) for eight AMR genes (*ant(6)-Ia*, *blaZ*, *dfrG*, *lnu(B)*, *lsa(E)*, *erm(C)*, *tet(M)* and *tet(K)*). Not submitting any of these chromosomal mutations or genes, and/or the respective predicted AMR phenotype, was scored with a zero. There were controversies regarding one hit:

Similarly to strain GENOMIC22-001, the *mecA* gene (hit in database: NC_007168) was identified in the sequence with 99,90% identity and 100,0% coverage. There are two base substitutions at positions 180 and 682 of the reference gene: codon GTT in reference gene becomes GTA in the genome (synonymous

mutation) and codon ACA in the reference gene becomes GCA in the genome, expected to lead to an amino acid substitution at position 228 of the protein (reference: WP_063852638) from A to T. This is a transition from a hydrophobic to a polar, non-charged amino acid. Based on the protein structure of penicillin-binding protein 2a (PBP2a) from methicillin-resistant *S. aureus* provided in Lim and Strynadka, 2022[2], this amino acid is expected to be in the non-penicillin binding domain of the protein, therefore not at the transpeptidase domain. Based on this and the fact that the strain is phenotypically resistant to FOX, it is expected that the protein is functional in this strain and confers resistance, despite the single amino acid substitution. Therefore, participants that did not submit the *mecA* gene were scored with a zero.

Table 5. AMR chromosomal mutations identified by the EURL-AR pipeline for S. aureus strain GENOMIC22-002 and the predicted AMR phenotypes.

Antimicrobial class	Gene	AMR Chrom. mutation	Predicted AMR Phenotype
Quinolone	*grlA*	S80Y	CIP
	gyrA	S84L	

Table 6. AMR genes identified by the EURL-AR pipeline for S. aureus strain GENOMIC22-002 and the respective predicted AMR phenotypes. Details regarding the hits in the ResFinder database as well as the percent identity (%ID) and coverage (%COV) to the reference gene are presented (hits from closed genomes).

Antimicrobial class	AMR Gene	Predicted AMR Phenotype (relevant for Gen. PT 2022)	Hit in database (Accession number)	Location	Position	%ID	%COV	Score if gene or predicted phenotype not reported
Aminoglycoside	*ant(6)-Ia*	STR	KF421157	chromosome	2173541..2174404	100.00	100.0	0
Beta-lactam	*blaZ*	PEN	NC_013374	chromosome	2099543..2100388	100.00	100.0	0
	mecA	FOX	NC_007168	chromosome	47597..49603	99.90	100.0	0
Folate pathway antagonist	*dfrG*	TMP	AB205645	chromosome	881780..882277	100.00	100.0	0
Lincosamide	*lnu(B)*	CLI	JQ861959	chromosome	2167621..2168424	100.00	100.0	0
Lincosamide, Streptogramin A, Pleuromutilin	*lsa(E)*	CLI, TIA	JX560992	chromosome	2168478..2169962	100.00	100.0	0
Macrolide, Lincosamide, Streptogramin B	*erm(C)*	CLI, ERY	M13761	plasmid_1	297..1031	100.00	100.0	0
Tetracycline	*tet(K)*	TET	U38656	chromosome	61587..62966	100.00	100.0	0
	tet(M)	TET	AM990992	chromosome	1063435..1065354	100.00	100.0	0

[2] Lim, D., Strynadka, N. Structural basis for the β lactam resistance of PBP2a from methicillin-resistant *Staphylococcus aureus*. *Nat Struct Mol Biol* **9**, 870–876 (2002). https://doi.org/10.1038/nsb858

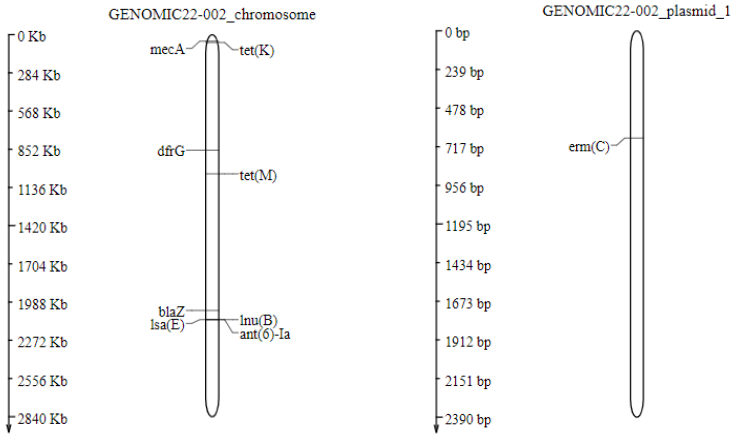

Figure 6. Schematic presentation of the location of the AMR genes identified by the EURL-AR pipeline for S. aureus strain GENOMIC22-002. The figure was generated using the online genetic map drawing tool MG2C (http://mg2c.iask.in/mg2c_v2.0/).

From the 25 participating laboratories, 23 analysed GENOMIC22-002-BACT and 22 GENOMIC22-002-DNA (see Table 3). An overview of the submitted AMR chromosomal mutations, AMR genes and the predicted AMR phenotype is presented in Figure 7, Figure 8, Figure 9 and Figure 10. The majority of participants identified and reported the expected AMR chromosomal mutations, as well as predicted phenotypic resistance to CIP. For the participants who did not report the expected mutations, EURL-AR identified them in their submitted DNA sequences, using PointFinder. Three participants (2022-01, 2022-10 and 2022-17) submitted different chromosomal mutations, probably due to typing mistake. These unexpected mutations were not identified by the EURL-AR pipeline in the participants' submitted sequences and were scored with a zero.

Regarding the nine expected AMR genes (*ant(6)-Ia*, *blaZ*, *mecA*, *dfrG*, *lnu(B)*, *lsa(E)*, *erm(C)*, *tet(M)*, *tet(K)*), the majority of the participants reported them in both BACT and DNA samples, as well as the respective predicted AMR phenotype (individual antimicrobials); however, only 10 (BACT) and 11 (DNA) participants submitted the expected predicted AMR profile. For the participants who did not report the expected genes, EURL-AR identified them in the participants' submitted DNA sequences, using ResFinder. A few unexpected genes were reported:

1) Gene *aadD* was reported by 16 participants: the gene is identified in the sequence by the EURL-AR pipeline too, but it should not have been reported. This gene is predicted to confer resistance to amikacin and tobramycin, which are not included in the antimicrobials relevant for *S. aureus* in Genomic PT 2022 (see Table 2). Reporting of this gene was scored with a zero.

2) Genes *aph(4)-Ia*, *erm(50)*, *lsa(B)*, *ant(4')-Ib*, *blaL*, *dfrB1*, *mph(A)* and *dfrD*, were reported by one or two participants each, but they were not identified by the EURL-AR pipeline. Reporting of these genes received a score ´0´.

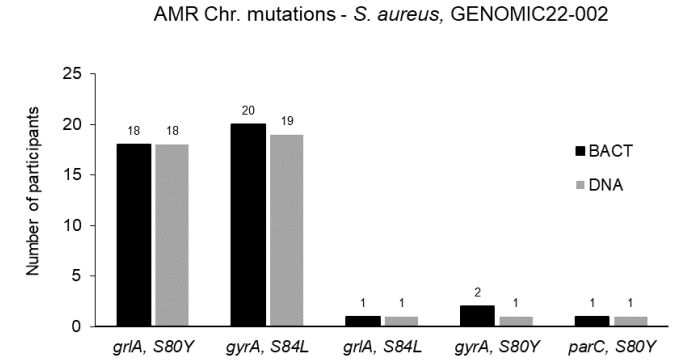

Figure 7. Overview of submitted AMR chromosomal mutations for S. aureus strain GENOMIC22-002.

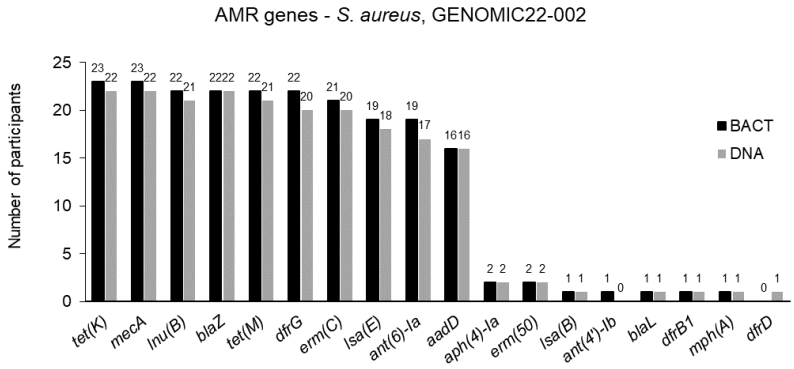

Figure 8. Overview of submitted AMR genes for S. aureus strain GENOMIC22-002.

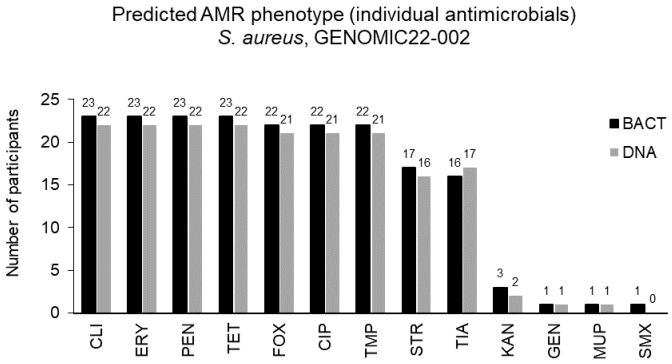

Figure 9. Overview of submitted predicted AMR phenotypes (individual antimicrobials) for S. aureus strain GENOMIC22-002.

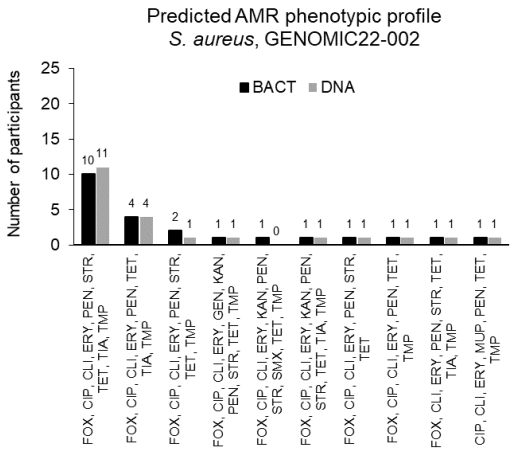

Figure 10. Overview of submitted predicted AMR profiles for S. aureus strain GENOMIC22-002.

6.3. *E. coli*, GENOMIC22-003

The AMR chromosomal mutations and genes as well as the potential predicted AMR phenotypes identified by the EURL-AR pipeline for strain GENOMIC22-003 are presented in Table 7 and

Table 8. Moreover, a schematic presentation of the location of the idenitified AMR genes is presented in Figure 11. The EURL-AR pipeline identified four chromosomal mutations in *gyrA*, *parC* and *parE* genes, potentially predicting a resistant phenotype to NAL and CIP. Moreover, seven AMR genes were identified (*bla*$_{NDM-5}$, *bla*$_{CTX-M-15}$, *bla*$_{TEM-1B}$, *sul1*, *dfrA12*, *mph(A)* and *tet(B)*) as perfect hits to reference genes of the ResFinder database (100,00% identity and 100,0% coverage of the reference gene). Not submitting any of the above-mentioned chromosomal mutations or genes, and/or the respective predicted AMR phenotype, was scored with a zero. There were controversies regarding one hit:

The *qepA4* gene (database hit: KX580704) was identified in the sequence with 99,93% identity and 100,0% coverage. There is a single nucleotide substitution at position 1487 of the reference gene: codon GGG becomes GGA in the genome. Both codons code for GLY, therefore the mutation is synonymous and it is not predicted to impact the protein structure or functionality. Consequently, participants that did not submit the *qepA4* gene received a zero score.

Table 7. AMR chromosomal mutations identified by the EURL-AR pipeline for E. coli strain GENOMIC22-003 and the predicted AMR phenotypes.

Antimicrobial class	Gene	AMR Chrom. mutation	Predicted AMR Phenotype (relevant for GENOMIC PT 2022)
Quinolone	*gyrA*	S83L	NAL, CIP
		D87N	
	parC	S80I	
	parE	S458A	

Table 8. AMR genes identified by the EURL-AR pipeline for E. coli strain GENOMIC22-003 and the respective predicted AMR phenotypes. Details regarding the hits in the ResFinder database as well as the percent identity (%ID) and coverage (%COV) to the reference gene are presented (hits from closed genomes). Note[1]: tet(B) was identified on plasmid 2 too at position 52072..53277.

Antimicrobial class	AMR Gene	Predicted AMR Phenotype (relevant for Gen. PT 2022)	Hit in database (Accession number)	Location	Position	%ID	%COV	Score if gene or predicted phenotype not reported
Beta-lactam	*bla*$_{CTX-M-15}$	AMP, FEP, FOT, TAZ	AY044436	chromosome	4025447..4026322	100.00	100.0	0
	bla$_{NDM-5}$	AMP, ETP, FEP, FOT, FOX, IMI, MERO, TAZ, TRM	JN104597	chromosome	104368..105180	100.00	100.0	0
	bla$_{TEM-1B}$	AMP	AY458016	plasmid_2	97100..97960	100.00	100.0	0
Folate pathway antagonist	*dfrA12*	TMP	AM040708	plasmid_2	112036..112533	100.00	100.0	0
	sul1	SMX	U12338	plasmid_2	109493..110332	100.00	100.0	0
Macrolide	*mph(A)*	AZI	D16251	plasmid_2	102078..102983	100.00	100.0	0
Quinolone	*qepA4*	CIP	KX580704	plasmid_2	115743..117278	99.93	100.0	0
Tetracycline	*tet(B)*	TET	AF326777	chromosome[1]	1919770..1920975	100.00	100.0	0

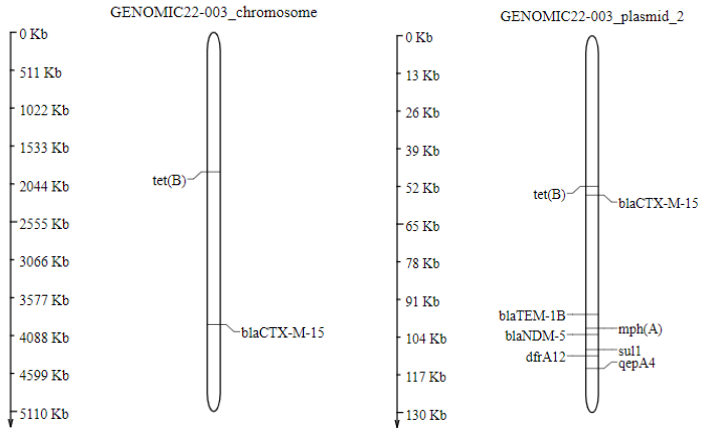

Figure 11. Schematic presentation of the location of the AMR genes identified by the EURL-AR pipeline, for E. coli strain GENOMIC22-003. The figure was generated using the online genetic map drawing tool MG2C (http://mg2c.iask.in/mg2c_v2.0/).

From the 25 participating laboratories, 25 analysed GENOMIC22-003-BACT and 24 GENOMIC22-003-DNA (see Table 3). An overview of the submitted AMR chromosomal mutations, AMR genes and the predicted AMR phenotype is presented in Figure 12, Figure 13, Figure 14 and Figure 15. The majority of participants identified and reported the expected AMR chromosomal mutations, as well as predicted phenotypic resistance to CIP and NAL. For the participants who did not report the expected mutations, EURL-AR identified them in their submitted DNA sequences, using PointFinder. Five unexpected mutations were reported by one participant each; however, they seem to be due to typing mistake, or because of reporting the mutations at a DNA level, instead of amino acid level, that was requested according to the Genomic PT 2022 protocol. Reporting of these unexpected chromosomal mutations received a score ´0´.

Regarding the eight expected AMR genes (*bla*$_{\text{NDM-5}}$, *bla*$_{\text{CTX-M-15}}$, *bla*$_{\text{TEM-1B}}$, *sul1*, *dfrA12*, *mph(A)*, *qepA4* and *tet(B)*), most of the participants reported them in both BACT and DNA samples, as well as the respective predicted AMR phenotype (individual antimicrobials); however, 18 (BACT) and 17 (DNA) participants submitted the expected predicted AMR profile. For the participants who did not report the expected genes, EURL-AR identified them in the submitted DNA sequences of these participants, using ResFinder. A few unexpected genes were reported:

1) Gene *aadA2* was reported by 15 (BACT) and 16 (DNA) participants: the gene is identified in the sequence by the EURL-AR pipeline too, but it should not have been reported. This gene potentially predicts resistance to spectinomycin and streptomycin, which are not included in the antimicrobials relevant for *E. coli* in the Genomic PT 2022 (see Table 2). Reporting of this gene was scored with a zero.

2) Genes *aadA8b*, *sul3*, *qepA1* and *qepA* were reported each by one participant, but they were not identified by the EURL-AR pipeline, in the participants' submitted sequences. Reporting of these genes received a score ´0´.

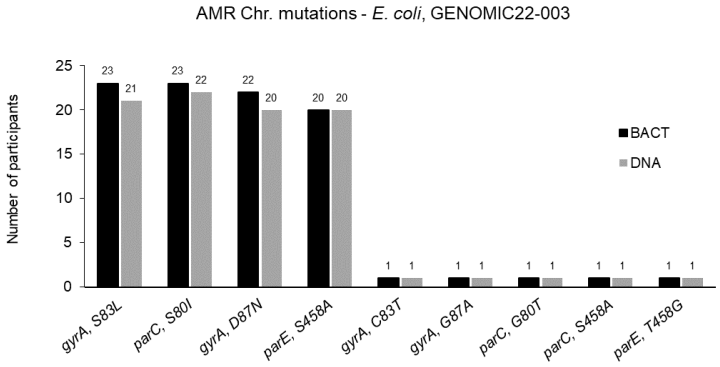

Figure 12. Overview of submitted AMR chromosomal mutations for E. coli strain GENOMIC22-003.

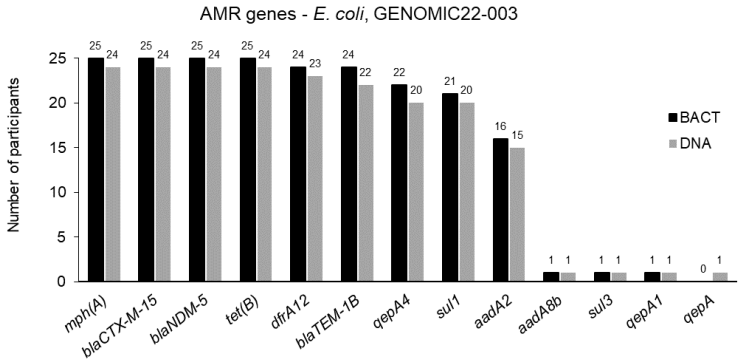

Figure 13. Overview of submitted AMR genes for E. coli, strain GENOMIC22-003.

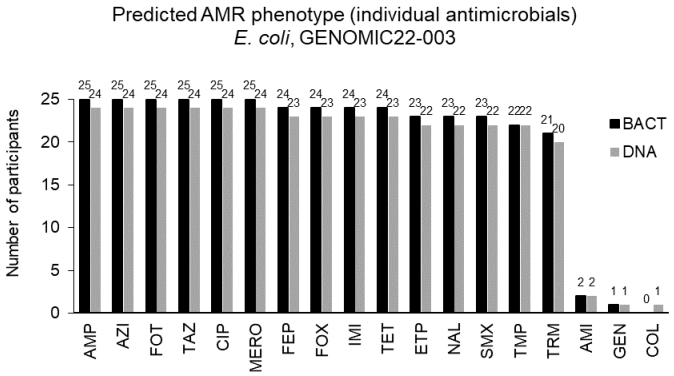

Figure 14. Overview of submitted predicted AMR phenotypes (individual antimicrobials) for E. coli strain GENOMIC22-003.

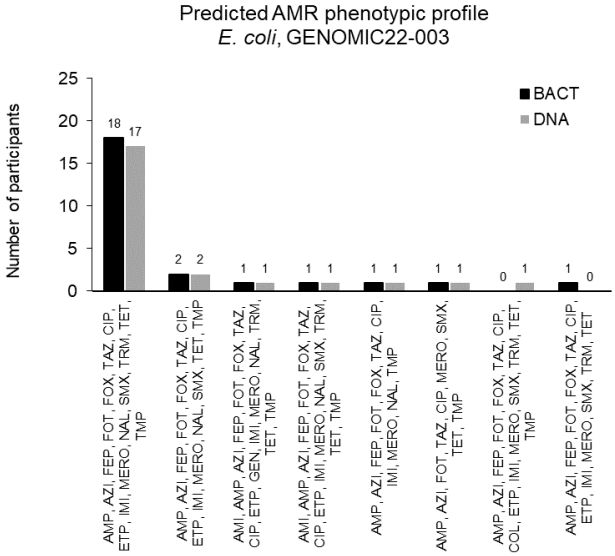

Figure 15. Overview of submitted predicted AMR profiles for E. coli strain GENOMIC22-003.

6.4. *E. coli*, GENOMIC22-004

The AMR chromosomal mutations and genes as well as the predicted AMR phenotypes identified by the EURL-AR pipeline for strain GENOMIC22-004 are presented in Table 9 and Table 10. Moreover, a schematic presentation of the location of the idenitified AMR genes is presented in Figure 16. The EURL-AR pipeline identified four chromosomal mutations in *gyrA*, *parC* and *parE* genes, predicting to confer resistance to NAL and CIP. Moreover, seven AMR genes were identified (*rmtC*, *aac(6')-Ib3*, *bla*$_{\text{CMY-6}}$, *bla*$_{\text{OXA-181}}$, *bla*$_{\text{NDM-1}}$, *sul1* and *qnrS1*) as perfect hits to reference genes of the ResFinder database (100,00% identity and 100,0% coverage of the reference gene). Not submitting any of the above-mentioned chromosomal mutations or genes, and/or the respective predicted AMR phenotype, was scored with a zero. There were controversies regarding one hit:

- The *aac(6')-Ib-cr* gene (hit in database: EF636461) was identified in the sequence with 99,61% identity and 100,0% coverage, at exactly the same position as gene *aac(6')-Ib3*, which is a perfect hit. There are two nucleotide substitution at positions 223 and 454 of the reference gene: codons AGG and TAT in the reference become TGG and GAT in the genome, respectively. These mutations lead to two amino acid substitutions from R (positively charged) to tryptophan (hydrophobic) at position 75 and from tyrosine (hydrophobic) to aspartic acid (negatively charged) at position 152 of the reference protein (ABV25531.1). The new amino acids have very different chemical properties compared to the ones in the reference protein, and their potential impact on the protein structure and functionality is unknown. Therefore, it was decided to score with a '1' if the gene was reported but blank the score if the gene was not reported. Reporting or not reporting of this gene has no impact on the predicted AMR phenotype, because resistance to CIP is mediated by other genes and chromosomal mutations in this strain.

Table 9. AMR chromosomal mutations identified by the EURL-AR pipeline for E. coli strain GENOMIC22-004 and the predicted AMR phenotypes.

Antimicrobial class	Gene	AMR Chrom. mutation	Predicted AMR Phenotype (relevant for GENOMIC PT 2022)
Quinolone	*gyrA*	S83L	CIP, NAL
		D87N	
	parC	S80I	
	parE	S458A	

Table 10. AMR genes identified by the EURL-AR pipeline for E. coli strain GENOMIC22-004 and the respective predicted AMR phenotypes. Details regarding the hits in the ResFinder database as well as the percent identity (%ID) and coverage (%COV) to the reference gene are presented (hits from closed genomes).

Antimicrobial class	AMR Gene	Predicted AMR Phenotype (relevant for Gen. PT 2022)	Hit in database (Accession number)	Location	Position	%ID	%COV	Score if gene or predicted phenotype not reported
Aminoglycoside	*aac(6')-Ib3*	AMI	X60321	Plasmid 1	38130..38684	100.00	100.0	0
	rmtC	AMI, GEN	AB194779	Plasmid 1	32922..33767	100.00	100.0	0
Beta-lactam	*bla*$_{\text{CMY-6}}$	AMP, FOT, FOX, TAZ	AJ011293	Plasmid 1	65396..66541	100.00	100.0	0

Antimicrobial class	AMR Gene	Predicted AMR Phenotype (relevant for Gen. PT 2022)	Hit in database (Accession number)	Location	Position	%ID	%COV	Score if gene or predicted phenotype not reported
	bla$_{NDM-1}$	AMP, FEP, FOT, FOX, TAZ, ETP, IMI, MERO, TRM	FN396876	Plasmid 1	30905..31717	100.00	100.0	0
	bla$_{OXA-181}$	AMP, FEP, ETP, IMI, MERO	CM004561	Plasmid 3	32054..32851	100.00	100.0	0
Folate pathway antagonist	*sul1*	SMX	U12338	Plasmid 1	36781..37620	100.00	100.0	0
Quinolone	*aac(6')-Ib-cr*	CIP	EF636461	Plasmid 1	38130..38648	99.61	100.0	**Blank**
	qnrS1	CIP	AB187515	Plasmid 3	38810..39466	100.00	100.0	0

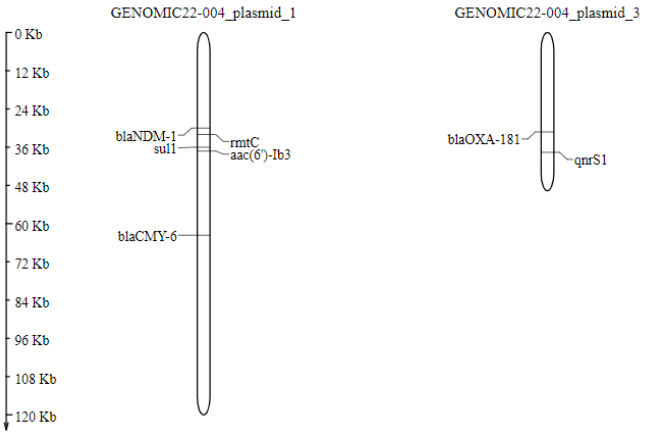

Figure 16. Schematic presentation of the location of the AMR genes identified by the EURL-AR pipeline, for E. coli strain GENOMIC22-004. The figure was generated using the online genetic map drawing tool MG2C (http://mg2c.iask.in/mg2c_v2.0/).

From the 25 participating laboratories, 25 analysed GENOMIC22-004-BACT and 24 GENOMIC22-004-DNA (see Table 3). An overview of the submitted AMR chromosomal mutations, AMR genes and the predicted AMR phenotype is presented in Figure 17, Figure 18, Figure 19 and Figure 20. The majority of participants identified and reported the expected AMR chromosomal mutations, as well as predicted phenotypic resistance to CIP and NAL. For the participants who did not report the expected mutations, EURL-AR identified them in their submitted DNA sequences, using PointFinder. Similarly to strain GENOMIC22-003, five unexpected mutations were reported by one participant each; however they seem to be due to typing mistake, or because of reporting the mutations at a DNA level, instead of amino acid level, as was requested in the GENOMIC PT 2022 protocol. Reporting of these unexpected chromosomal mutations received a score zero.

Regarding the eight expected AMR genes (*rmtC*, *aac(6')-Ib3*, *aac(6')-Ib-cr*, *bla*$_{CMY-6}$, *bla*$_{OXA-181}$, *bla*$_{NDM-1}$, *sul1* and *qnrS1*), most of the participants reported them in both BACT and DNA samples, as well as the

respective predicted AMR phenotype (individual antimicrobials); however, 18 (BACT) and 16 (DNA) participants submitted the expected predicted AMR profile. For the participants who did not report the expected genes, EURL-AR identified them in the submitted DNA sequences of these participants, using ResFinder. A few unexpected genes were reported by a few participants:

- Genes *aac(6')-Ib*, *aac(6')-Ib11* and *sul3* were not identified by the EURL-AR pipeline in the participants's submitted sequences. Reporting of these genes received a score ´0´.

- Gene *aac(6´)lb-cr*, is likely a typo for aac(6´)-lb-cr, but still received a score ´0´ due to incorrect reporting.

- Gene *erm(C)*, reported by laboratory 2022-15 in the DNA sample is identified by the EURL-AR pipeline too in the submitted sequence of this laboratory, as a perfect hit. There is no hit for *erm(C)* in the original closed or assembled genomes prepared by the EURL-AR, or the raw reads. We cannot be sure why that happened. It could be a contamination of the DNA sample for this laboratory. The score for submitting gene *erm(C)* is zero.

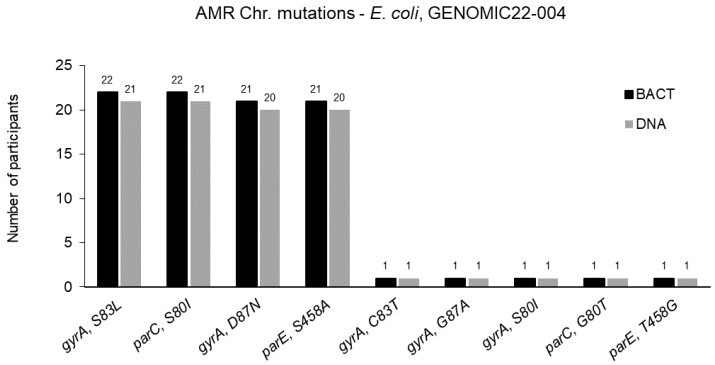

Figure 17. Overview of submitted AMR chromosomal mutations for E. coli strain GENOMIC22-004.

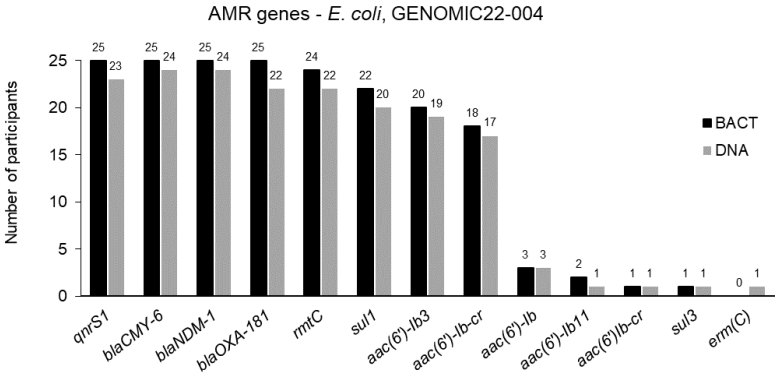

Figure 18. Overview of submitted AMR genes for E. coli, strain GENOMIC22-004.

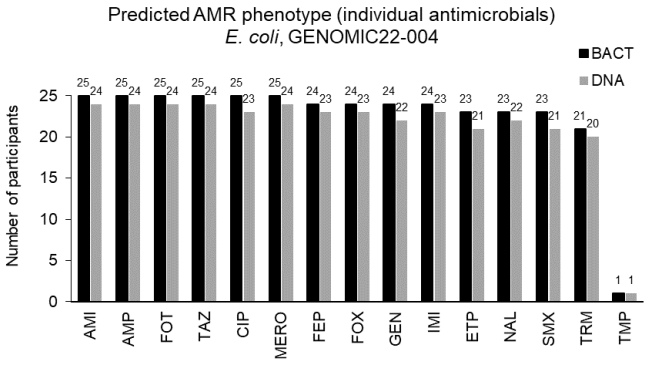

Figure 19. Overview of submitted predicted AMR phenotypes (individual antimicrobials) for E. coli strain GENOMIC22-004.

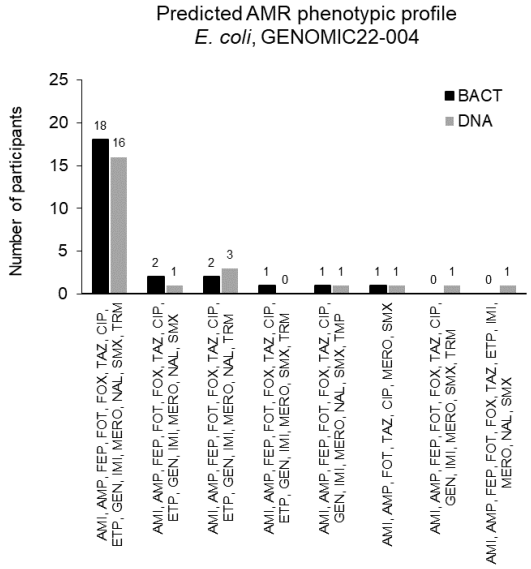

Figure 20. . Overview of submitted predicted AMR profiles for E. coli strain GENOMIC22-004.

6.5. *E. faecium*, GENOMIC22-005

The AMR chromosomal mutations and genes as well as the predicted AMR phenotypes identified by the EURL-AR pipeline for strain GENOMIC22-005 are presented in Table 13 and

Table 14. Moreover, a schematic presentation of the location of the idenitified AMR genes is presented in Figure 21. The EURL-AR pipeline identified 19 chromosomal mutations in *pbp5* gene, which collectively are predicted to confer resistance to AMP. Moreover, one mutation was identified in *gyrA* and one in *parC*, predicted to confer resistance to NAL and CIP. Three AMR genes were identified (*VanHAX*, *erm(A)* and *erm(B)*) as perfect hits to reference genes of the ResFinder database (100,00% identity and 100,0% coverage). Not submitting any of the above-mentioned chromosomal mutations or genes, and/or the respective predicted AMR phenotype, was scored with a zero. There were controversies regarding three hits:

1) The *aac(6')-Ii* gene (hit in database: L12710) was identified in the sequence with 99,64% identity and 100,0% coverage. There are two nucleotide substitution at positions 380 and 538 of the reference gene: codons GTG and GAT become GAG and AAT in the genome, respectively. These mutations lead to two amino acid substitutions from valine (hydrophobic) to E (negatively charged) at position 127 and from aspartic acid (negatively charged) to N (polar, uncharged) at position 180 of the reference protein (AAB63533.1). These are major changes chemically, and their potential impact on the protein structure and functionality is unknown. Therefore, it was decided to score

with a '1' if the gene was reported but blank the score if the gene was not reported. Predicted AMR profiles with or without GEN were accepted (score=1).

2) The *msr(C)* gene (hit in database: AY004350) was identified in the sequence with 98,92% identity and 100,0% coverage. There are 16 nucleotide substitution leading to 6 amino acid substitutions: a) proline to leucine, b) aspartic acid to glycine, c) valine to I, d) A to T, e) methionine to T and f) valine to I. These are major changes chemically, and their potential impact on the protein structure and functionality is unknown. Therefore, it was decided to score with a '1' if the gene was reported but blank the score if the gene was not reported. Reporting or not reporting of this gene has no impact on the predicted AMR phenotype, because resistance to ERY is mediated by other genes too in this strain.

3) The *tet(M)* gene (hit in database: FN433596) generated two hits in the genome, with 100,00% identity to the reference gene, but 58,8 and 41,7% coverage respectively. The two hits were coming from different positions in the closed genome – see Table 11 as well as the assembled genome (same contig though) - see Table 12. In the raw reads (fastq) there was one hit for *tet(M)* FN433596 with 100,00% identity but one nucleotide longer than the reference gene (one extra base pair in the middle of the sequence). Due to the above, it was decided to score with a '1' if the gene was reported but blank the score if the gene was not reported. Predicted AMR profiles with or without TET were accepted (score=1).

Table 11. Hits for tet(M) gene identified by the EURL-AR pipeline in the closed genome of E. faecium GENOMIC22-005.

Gene name	Reference gene ID	%ID	Length (%COV)	Contig name	Position
tet(M)	FN433596	100.00	1128/1920 (58.8)	chromosome	2831031..2832158
tet(M)	FN433596	100.00	801/1920 (41.7)	chromosome	2835435..2836235

Table 12. Hits for tet(M) gene identified by the EURL-AR pipeline in the assembled genome of E. faecium GENOMIC22-005.

Gene name	Reference gene ID	%ID	Length (%COV)	Contig name	Position
tet(M)	FN433596	100.00	1128/1920 (58.8)	NODE_3_length_96217_cov_27.1	19159..20286
tet(M)	FN433596	100.00	801/1920 (41.7)	NODE_3_length_96217_cov_27.1	15082..15882

Table 13. AMR chromosomal mutations identified by the EURL-AR pipeline for E. faecium strain GENOMIC22-005 and the predicted AMR phenotypes.

Antimicrobial class	Gene	AMR Chrom. mutation	Predicted AMR Phenotype (relevant for GENOMIC PT 2022)
Beta-lactam	*pbp5*	A216S, A499T, A68T, D204G, E100Q, E525D, E629V, E85D, G66E, K144Q, L177I, M485A, N496K, P667S, R34Q, S27G, T172A, T324A, V24A	AMP
Quinolone	*gyrA*	S83Y	CIP
	parC	S80I	CIP

Table 14. AMR genes identified by the EURL-AR pipeline for E. faecium strain GENOMIC22-005 and the respective predicted AMR phenotypes. Details regarding the hits in the ResFinder database as well as the percent identity (%ID) and coverage (%COV) to the reference gene are presented (hits from closed genomes). Note[1]: The tet(M) gene was split in two hits with 100% identity to the reference. Both hits were identified on the chromosome, at positions 2831031..2832158 and 2835435..2836235.

Antimicrobial class	**AMR Gene**	**Predicted AMR Phenotype (relevant for Gen. PT 2022)**	**Hit in database (Accession number)**	**Location**	**Position**	**%ID**	**%COV**	**Score if gene or predicted phenotype not reported**
Aminoglycoside	*aac(6')-Ii*	GEN	L12710	chromosome	2307853..2308401	99.64	100.0	**Blank**
Glycopeptide	*VanHAX*	TEI, VAN	M97297	plasmid_2	21432..24038	100.00	100.0	0
Macrolide, Streptogramin B	*msr(C)*	ERY	AY004350	chromosome	166703..168181	98.92	100.0	**Blank**
Macrolide, Lincosamide, Streptogramin_B	*erm(A)*	ERY	X03216	chromosome	2203261..2203992	100.00	100.0	0
	erm(B)	ERY	U18931	plasmid_2	31657..32394	100.00	100.0	0
Tetracycline	*tet(M)*	TET	FN433596	chromosome	Note[1]	100.00	Note[1]	**Blank**

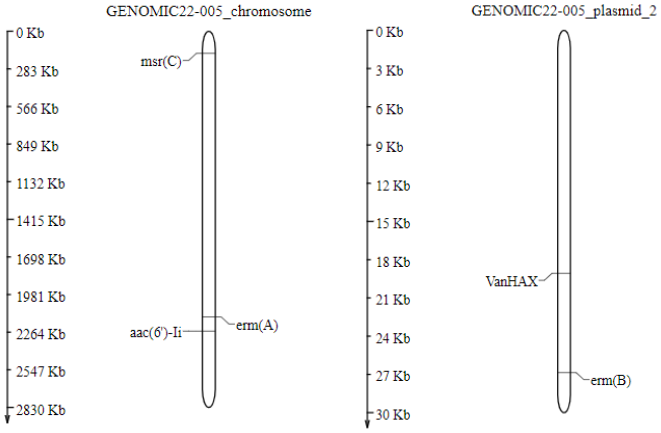

Figure 21. Schematic presentation of the location of the AMR genes identified by the EURL-AR pipeline, for E. faecium strain GENOMIC22-005. The figure was generated using the online genetic map drawing tool MG2C (http://mg2c.iask.in/mg2c_v2.0/).

From the 25 participating laboratories, 19 analysed GENOMIC22-005-BACT and 18 GENOMIC22-005-DNA (see Table 3). An overview of the submitted AMR chromosomal mutations, AMR genes and the predicted AMR phenotype is presented in Figure 22, Figure 23, Figure 24 and Figure 25. The majority of participants identified and reported the expected AMR chromosomal mutations, as well as predicted phenotypic resistance to AMP and CIP. For the participants who did not report the expected mutations, EURL-AR identified them in their submitted DNA sequences, using PointFinder. Five unexpected mutations were reported by one participant each; however, they were not identified by EURL-AR. Reporting of these unexpected chromosomal mutations, received a score ´0´.

Regarding the five expected AMR genes (*aac(6')-Ii*, *VanHAX*, *msr(C)*, *erm(A)* and *erm(B)*), the majority of the participants reported them in both BACT and DNA samples, as well as the respective predicted AMR phenotype (individual antimicrobials). The *tet(M)* gene, and the respective predicted resistance to

TET (see above for details on the issue with the *tet(M)* gene) were reported by more than half of the participants. The expected predicted AMR profile was reported by 14 participants. For the participants who did not report the expected genes, EURL-AR identified them in the submitted DNA sequences of these participants, using ResFinder. Unexpected genes were reported by a minority of the participants:

- Gene *ant(9)-Ia* was reported by 11(BACT) and 10 (DNA) participants, and was identified in the sequence by the EURL-AR pipeline too. However, *ant(9)-Ia* is predicted to confer phenotypic resistance to spectinomycin, which is not included in the list of antimicrobials for *Enterococcus* for GENOMIC PT 2022 (Table 2), so it should have not been reported. Reporting of *ant(9)-Ia* received a score ´0´.

- Gene *aac(6')-aph(2")*, reported by 7 participants, was a hit with low quality paramerters (%coverage <80) and therefore is regarded as a mistake (score=0).

- Gene *VanA* was reported by two participants, and it was decided to score it as correct (score=1). The new nomenclature for the *VanA* gene is *VanHAX*.

- Gene *VanH* and *VanX* are regulatory and not AMR genes, therefore should not have been reported. Reporting of these genes received a score ´0´.

Genes *erm(50)*, *aph(2")-Ia*, *tet(S/M)* and *str* were not identified by the EURL-AR pipeline, and reporting of these genes received a score zero.

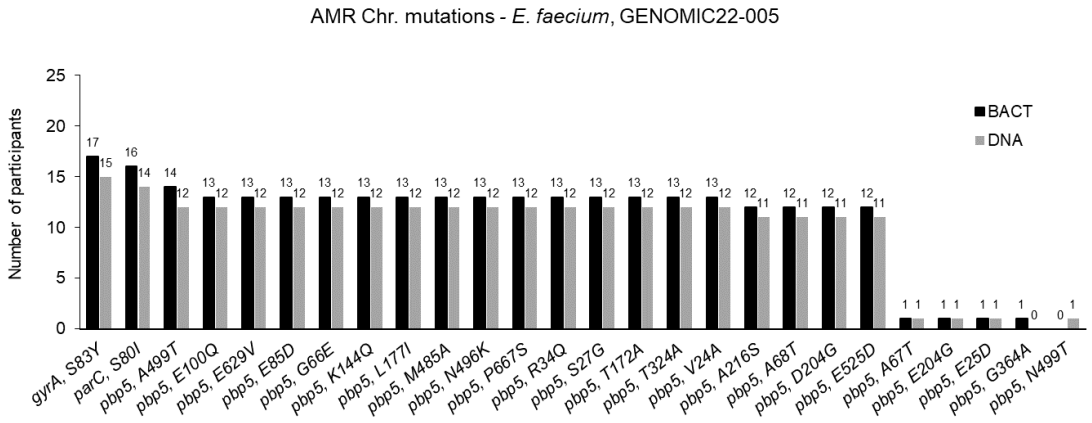

Figure 22. Overview of submitted AMR chromosomal mutations for E. faecium, strain GENOMIC22-005 (BACT and DNA samples).

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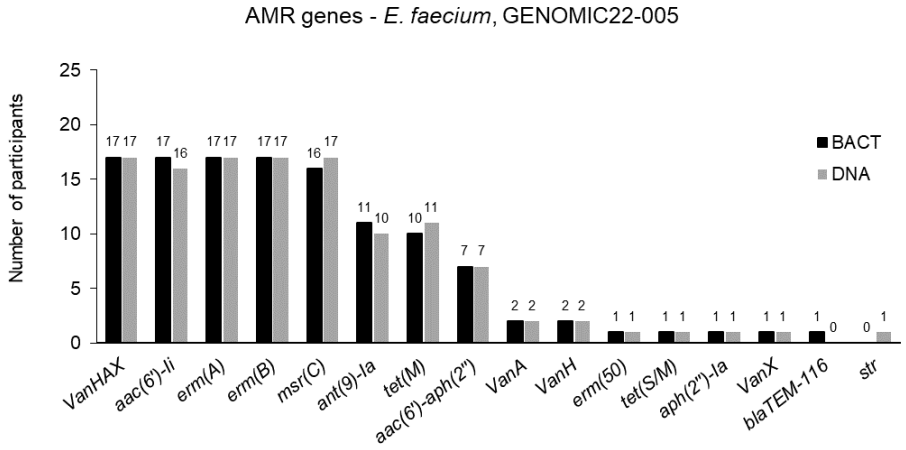

Figure 23. Overview of submitted AMR genes for E. faecium, strain GENOMIC22-005 (BACT and DNA samples).

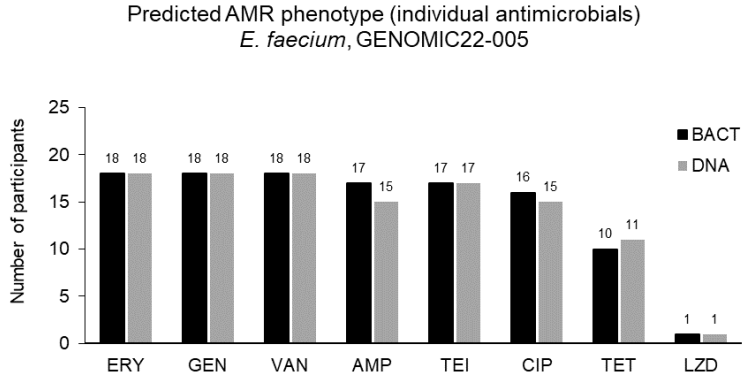

Figure 24. Overview of submitted predicted AMR phenotypes (individual antimicrobials) for E. faecium strain GENOMIC22-005.

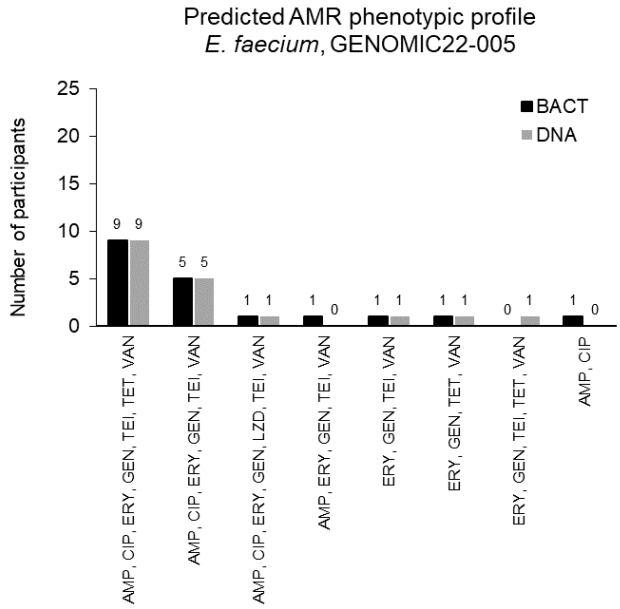

Figure 25.Overview of submitted predicted AMR profiles for E. faecium strain GENOMIC22-005.

6.6. *E. faecalis*, GENOMIC22-006

The AMR chromosomal mutations and genes as well as the predicted AMR phenotypes identified by the EURL-AR pipeline for strain GENOMIC22-006 are presented in Table 15 and Table 16. Moreover, a

schematic presentation of the location of the idenitified AMR genes is presented in Figure 26. The EURL-AR pipeline identified one chromosomal mutation in *parC* and one in *gyrA*, predicting phenotypic resistance to NAL and CIP. Three AMR genes were identified (*aac(6')-aph(2")*, *erm(B)* and *tet(M)*) as perfect hits to reference genes in the ResFinder database (100,00% identity and 100,0% coverage). Not submitting any of the above-mentioned chromosomal mutations or genes, and/or the respective predicted AMR phenotype, was scored with a zero. There were controversies regarding two hits:

1) The *cat(pC221)* gene (hit in database: X02529) was identified in the sequence with 97,69% identity and 100,0% coverage. There are 15 nucleotide substitution expected to result in 6 amino acid substitutions (protein reference CAA26367.1): a) position 7, K to E, b) position 106, N to K, c) position 109, T to I, d) position 161, N to S, e) position 190, A to S and f) position 210, K to R. The potential impact of these changes on the protein structure and functionality is unknown; therefore, it was decided to score with a '1' if the gene was reported but blank the score if the gene was not reported. Predicted AMR profiles with or without CHL were accepted (score=1).

2) The *VanHBX* gene (hit in database AF192329) was identified in the sequence (closed and assembled genome) with 98,5% identity and 99,9% coverage. There are several mutations leading to 21 amino acid substitutions at protein level and an amino acid addition. The potential effect of these changes in the protein structure and functionality is unknown, therefore it was decided to score with a '1' if the gene was reported but blank the score if the gene was not reported. Predicted AMR profiles with or without VAN were accepted (score=1).

Table 15. AMR chromosomal mutations identified by the EURL-AR pipeline for E. faecalis strain GENOMIC22-006 and the predicted AMR phenotype.

Antimicrobial class	Gene	AMR Chrom. mutation	Predicted AMR Phenotype
Quinolone	*parC*	S80I	CIP
	gyrA	S83I	CIP

Table 16. AMR genes identified by the EURL-AR pipeline for E. faecalis strain GENOMIC22-006 and the respective predicted AMR phenotypes. Details regarding the hits in the ResFinder database as well as the percent identity (%ID) and coverage (%COV) to the reference gene are presented (hits from closed genomes). Note[1]: erm(B) gene is identified on plasmid 3 too, as a perfect hit (position 52193..52930). Note[2]: tet(M) is identified at another position on the chromosome too as a perfect hit (position 346799..348718).

Antimicrobial class	AMR Gene	Predicted AMR phenotype (relevant for Gen. PT 2022)	Hit in database (Accession number)	Location	Position	%ID	%COV	Score if gene or predicted phenotype not reported
Aminoglycoside	*aac(6')-aph(2")*	GEN	M13771	chromosome	2824718..2826157	100.00	100.0	0
Amphenicol	*cat(pC221)*	CHL	X02529	plasmid_1	34561..35208	97.69	100.0	**Blank**
Glycopeptide	*VanH*BX	VAN	AF192329	chromosome	2935626..2938232	98.54	99.9	**Blank**
Macrolide, Lincosamide, Streptogramin_B	*erm(B)*[1]	ERY	U86375	plasmid_1	38616..39353	100.00	100.0	0
Tetracycline	*tet(M)*[2]	TET	AM990992	chromosome	661721..663640	100.00	100.0	0

From the 25 participating laboratories, 19 analysed GENOMIC22-006-BACT and 18 GENOMIC22-006-DNA (see Table 3). An overview of the submitted AMR chromosomal mutations, AMR genes and the predicted AMR phenotype is presented in Figure 27, Figure 28, Figure 29 and Figure 30. The majority of participants identified and reported the expected AMR chromosomal mutations, as well as predicted phenotypic resistance to CIP. For the participants who did not report the expected chromosomal mutations, EURL-AR identified them in the submitted DNA sequences of these participants, using PointFinder.

Regarding the five expected AMR genes (*aac(6')-aph(2")*, *erm(B)*, *cat(pC221)*, *VanHBX* and *tet(M)*), the majority of the participants reported them in both BACT and DNA samples, as well as the respective predicted AMR phenotype (individual antimicrobials). The expected predicted AMR profile was reported by 9 (BACT) and 8 (DNA) participants (profiles with or without VAN and/or CHL resistance were accepted). For the participants who did not report the expected genes, EURL-AR identified them in the submitted DNA sequences of these participants, using ResFinder. Unexpected genes were reported by a minority of the participants:

- Gene *cat* was reported by 4 participants, however this was not identified by the EURL-AR pipeline. We speculate that the intention of the participants was to report *cat(pC221)* instead of *cat*. Reporting of the gene cat received a score ´0´.
- Genes *ant(9)-Ia*, *erm(A)*, *aph(2")-Ia* and *VanHAX* were not identified by the EURL-AR pipeline, and reporting of these genes received a score ´0´.
- Gene *erm(C)* was reported by laboratory 2022-15 (DNA), and indeed a perfect hit for *erm(C)* was idenitified by the EURL-AR pipeline in their submitted sequence too. There is no hit for *erm(C)* in the original closed or assembled genomes prepared by the EURL-AR, or the raw reads. Laboratory 2022-15 reported the *erm(C)* gene in the DNA samples for strains GENOMIC22-001, GENOMIC22-004 and GENOMIC22-006 as well as the BACT sample for GENOMIC22-001. This could be a systemic error maybe due to contamination. However, the fact that it was identified in both BACT and DNA samples, makes things even more complicated. Due to the fact that *erm(C)* gene was not identified in the sequences generated by the EURL-AR, and also only one participant reported it, was scored with a zero.

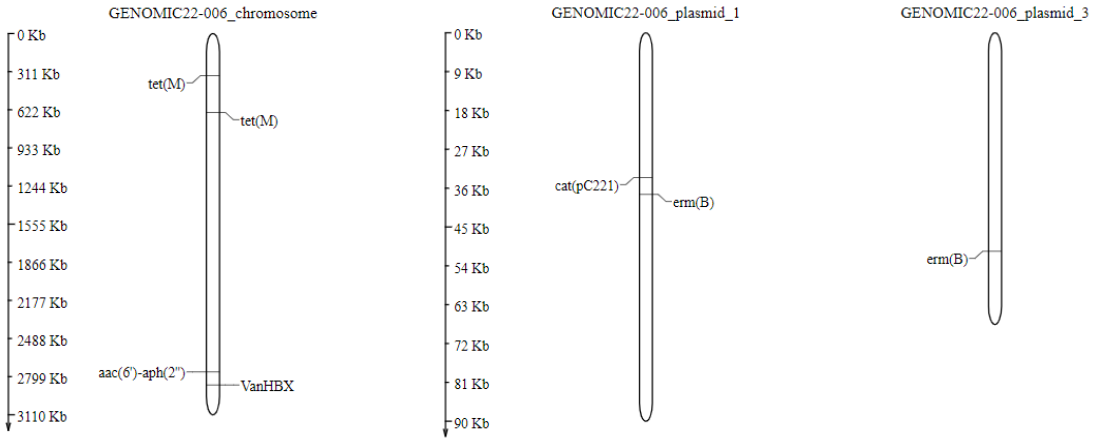

Figure 26. Schematic presentation of the location of the AMR genes, identified by the EURL-AR pipeline, for E. faecalis strain GENOMIC22-006. The tet(M) gene was identified in two copies on the bacterial chromosome. The erm(B) gene was identified in two copies, on two different plasmids. The figure was generated using the online genetic map drawing tool MG2C (http://mg2c.iask.in/mg2c_v2.0/).

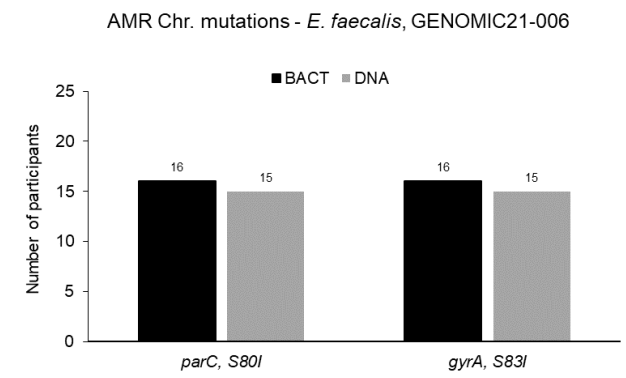

Figure 27. Overview of submitted AMR chromosomal mutations for E. faecalis strain GENOMIC22-006.

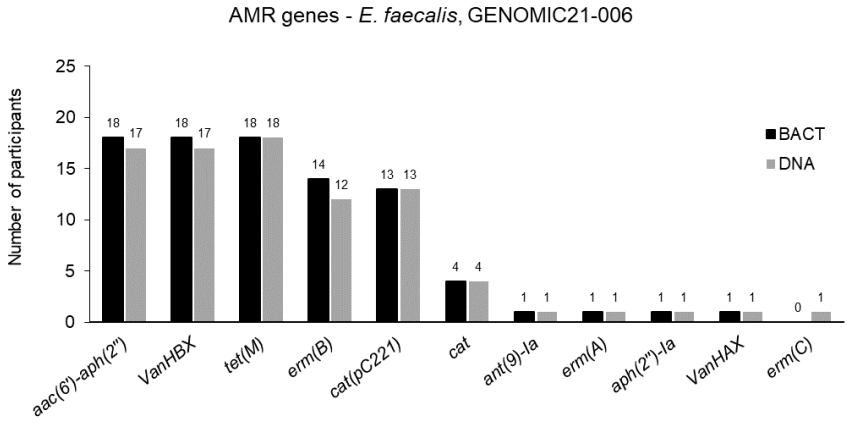

Figure 28. Overview of submitted AMR genes for E. faecalis strain GENOMIC22-006.

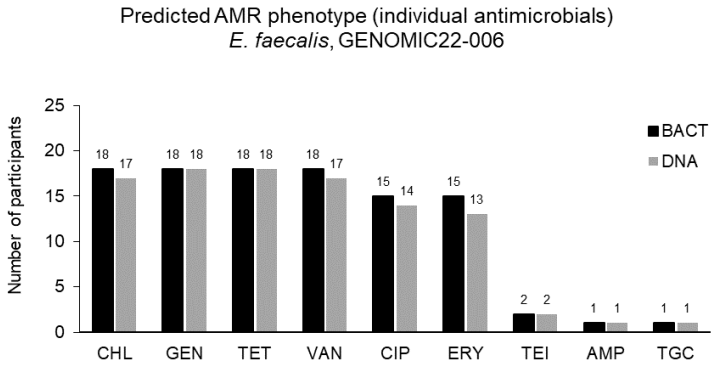

Figure 29. Overview of submitted predicted AMR phenotypes (individual antimicrobials) for E. faecalis strain GENOMIC22-006.

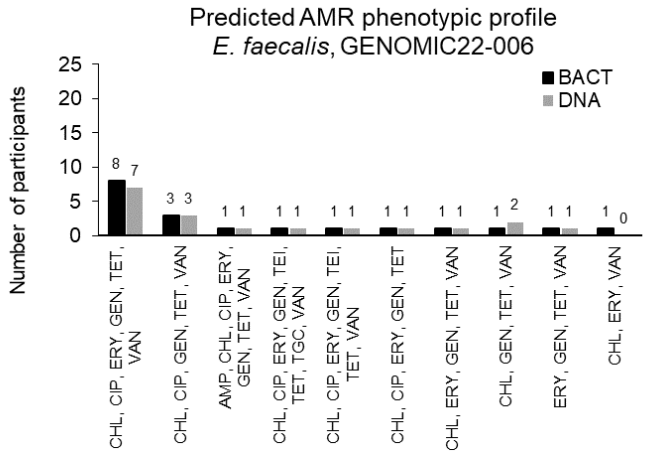

Figure 30. Overview of submitted predicted AMR profiles for E. faecalis strain GENOMIC22-006.

7. PARTICIPANTS' PERFORMANCE - AMR

The sum of scores for each laboratory as well as the maximum score that could be obtained per AMR category is presented in Table 17 for AMR chromosomal mutations, in Table 18 for AMR genes and in Table 19 for the predicted AMR phenotype (individual antibiotics). The average performance of the participants for all strains was expressed as percent of the maximum score that could be obtained for each category and is presented in Figure 31 (AMR chromosomal mutations), Figure 32 (AMR genes) and Figure 33 (predicted AMR phenotype).

Table 17. Maximum score, and sum of scores per participant for AMR chromosomal mutations, for each sample. n/a: laboratory did not participate.

		Max score	2022-01	2022-02	2022-03	2022-04	2022-05	2022-06	2022-07	2022-08	2022-09	2022-10	2022-11	2022-12	2022-13	2022-14	2022-15	2022-16	2022-17	2022-18	2022-19	2022-21	2022-22	2022-23	2022-24	2022-25	2022-26
GENOMIC22-002	BACT	**2**	1	2	2	n/a	2	2	2	n/a	2	0	2	2	2	2	2	0	1	0	2	2	2	2	2	2	2
	DNA	**2**	2	n/a	2	n/a	2	2	2	n/a	2	0	2	2	2	2	2	0	1	0	2	2	2	2	2	2	2
GENOMIC22-003	BACT	**4**	4	4	4	3	3	4	4	0	4	4	4	4	4	4	4	0	4	4	4	4	4	4	3	3	4
	DNA	**4**	4	n/a	4	3	3	4	4	1	4	4	4	4	4	4	4	0	4	4	4	4	4	4	3	1	4
GENOMIC22-004	BACT	**4**	4	4	4	3	4	4	4	0	4	3	4	4	4	4	4	0	4	4	4	4	4	4	3	1	4
	DNA	**4**	4	n/a	4	3	4	4	4	0	4	3	4	4	4	4	4	0	4	4	4	4	4	4	3	1	4
GENOMIC22-005	BACT	**21**	21	21	2	n/a	0	3	21	n/a	21	19	n/a	n/a	21	21	21	2	21	0	21	21	20	2	n/a	n/a	19
	DNA	**21**	21	n/a	2	n/a	0	3	21	n/a	21	19	n/a	n/a	21	21	21	0	21	0	21	20	20	2	n/a	n/a	19
GENOMIC22-006	BACT	**2**	2	2	2	n/a	2	2	2	n/a	2	2	n/a	n/a	2	2	0	0	2	0	2	2	2	2	n/a	n/a	2
	DNA	**2**	2	n/a	2	n/a	2	2	2	n/a	2	2	n/a	n/a	2	2	0	0	2	0	2	2	2	2	n/a	n/a	2

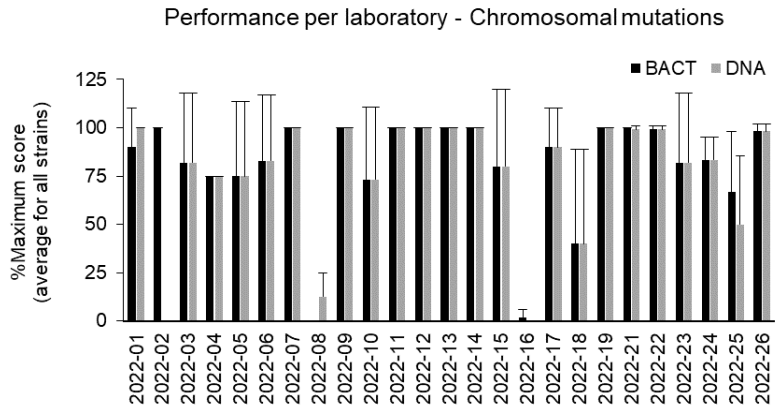

Figure 31. Performance of participants regarding the identification of expected AMR chromosomal mutations, expressed as percent of the maximum score. Average of all strains and standard deviation are plotted.

Table 18. Maximum score, and sum of scores per participant for AMR genes, for each sample. n/a: laboratory did not participate. (1) Scores for reporting genes lsa(B) and lsa(E) for strain *GENOMIC22-001 were subtracted from the maximum score, (2) Scores for reporting gene aac(6')-Ib-cr for* strain *GENOMIC22-004 were subtracted from the maximum score, (3) Scores for reporting genes aac(6')-Ii, msr(C) and tet(M) for* strain *GENOMIC22-005 were subtracted from the maximum score, (4) Scores for reporting genes cat(pC221) and VanHBX for* strain *GENOMIC22-006 were subtracted from the maximum score.*

		Max score	2022-01	2022-02	2022-03	2022-04	2022-05	2022-06	2022-07	2022-08	2022-09	2022-10	2022-11	2022-12	2022-13	2022-14	2022-15	2022-16	2022-17	2022-18	2022-19	2022-21	2022-22	2022-23	2022-24	2022-25	2022-26
GENOMIC22-001	BACT	9(1)	9	7	8	n/a	9	9	9	n/a	7	8	7	9	9	9	6	8	9	8	9	8	9	9	9	5	9
	DNA	9(1)	9	n/a	9	n/a	9	9	9	n/a	7	9	9	9	9	9	6	8	9	8	9	8	9	9	9	5	9
GENOMIC22-002	BACT	9	9	9	7	n/a	8	9	9	n/a	9	9	9	9	9	9	7	8	9	9	8	8	9	9	7	5	9
	DNA	9	8	n/a	6	n/a	8	9	9	n/a	9	9	9	9	9	9	7	8	9	9	9	8	9	9	7	5	9
GENOMIC22-003	BACT	8	7	8	8	8	7	8	8	8	8	8	8	8	8	8	6	7	8	8	7	8	7	8	7	7	8
	DNA	8	7	n/a	8	8	7	8	8	8	8	8	8	8	8	8	5	7	8	8	7	8	7	8	7	6	8
GENOMIC22-004	BACT	8(2)	7	6	7	7	6	7	7	7	7	7	7	7	7	6	6	7	7	7	7	7	5	7	6	5	7
	DNA	8(2)	5	n/a	7	7	6	7	7	6	7	7	5	7	7	5	6	7	7	7	7	7	5	7	6	5	7
GENOMIC22-005	BACT	3(3)	1	3	1	n/a	3	3	3	n/a	3	3	n/a	n/a	3	3	3	0	3	3	3	3	3	3	n/a	n/a	3
	DNA	3(3)	3	n/a	1	n/a	3	3	3	n/a	3	3	n/a	n/a	3	3	3	3	3	3	3	3	3	3	n/a	n/a	3
GENOMIC22-006	BACT	3(4)	2	3	1	n/a	3	2	3	n/a	3	3	n/a	n/a	3	3	3	2	3	2	3	3	2	3	n/a	n/a	3
	DNA	3(4)	2	n/a	2	n/a	3	2	2	n/a	3	3	n/a	n/a	3	3	3	2	3	2	3	3	2	3	n/a	n/a	3

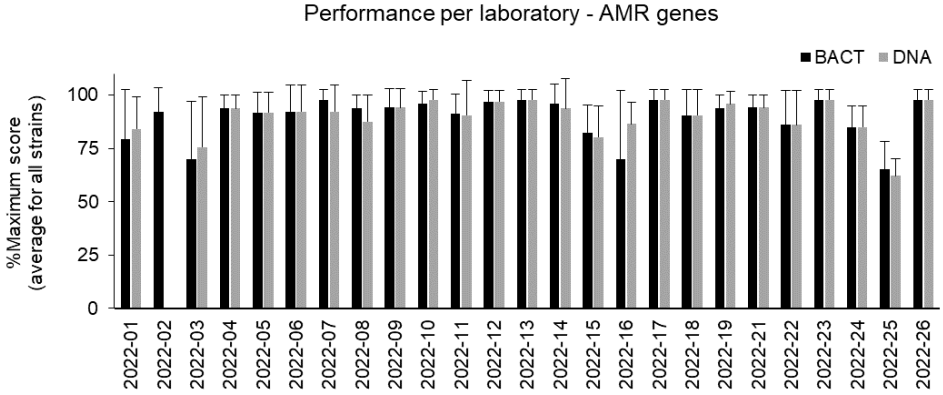

Figure 32. Performance of participants regarding the identification of expected AMR genes, expressed as percent of the maximum score. Average of all strains *and standard deviation are plotted.*

Table 19. Maximum score, and sum of scores per participant for predicted AMR phenotype, for each sample. n/a: laboratory did not participate. (1) Scores for reporting predicted resistance phenotype to GEN and TET for strain *GENOMIC22-005 were subtracted from the maximum score, (2) Scores for reporting predicted resistance phenotype to CHL and VAN for strain GENOMIC22-006 were subtracted from the maximum score.*

		Max score	2022-01	2022-02	2022-03	2022-04	2022-05	2022-06	2022-07	2022-08	2022-09	2022-10	2022-11	2022-12	2022-13	2022-14	2022-15	2022-16	2022-17	2022-18	2022-19	2022-21	2022-22	2022-23	2022-24	2022-25	2022-26
GENOMIC22-001	BACT	9	9	8	9	0	6	9	9	n/a	8	9	7	8	9	9	8	9	8	9	9	8	9	9	8	5	9
	DNA	9	9	n/a	8	0	6	9	9	n/a	8	9	9	8	9	9	8	9	8	9	9	8	9	9	8	5	9
GENOMIC22-002	BACT	7	7	6	7	0	6	7	7	n/a	7	7	7	7	7	7	6	7	6	6	7	6	7	7	6	5	7
	DNA	7	7	n/a	6	0	6	7	7	n/a	7	7	7	7	7	7	6	7	6	6	7	6	7	7	6	5	7
GENOMIC22-003	BACT	15	14	15	13	14	11	15	15	15	15	15	15	15	15	15	15	15	15	14	15	15	13	15	15	9	15
	DNA	15	14	n/a	14	14	11	15	15	15	15	15	15	15	15	15	15	15	15	14	15	15	13	15	15	9	15
GENOMIC22-004	BACT	14	13	14	13	14	12	14	14	14	14	14	14	14	14	14	14	13	14	13	14	14	13	14	14	7	14
	DNA	14	11	n/a	12	14	12	14	14	14	14	14	14	14	14	13	14	13	14	13	14	14	13	14	14	7	14
GENOMIC22-005	BACT	5	5	5	4	0	2	5	5	n/a	5	5	n/a	n/a	5	5	5	2	5	3	5	5	5	5	n/a	n/a	5
	DNA	5	5	n/a	3	0	2	5	5	n/a	5	5	n/a	n/a	5	5	5	5	5	3	5	5	5	5	n/a	n/a	5

		Max score	2022-01	2022-02	2022-03	2022-04	2022-05	2022-06	2022-07	2022-08	2022-09	2022-10	2022-11	2022-12	2022-13	2022-14	2022-15	2022-16	2022-17	2022-18	2022-19	2022-21	2022-22	2022-23	2022-24	2022-25	2022-26
GENOMIC22-006	BACT	**4**	3	3	1	0	4	3	4	n/a	4	4	n/a	n/a	4	4	3	3	4	2	4	4	4	4	n/a	n/a	4
	DNA	**4**	3	n/a	2	0	4	3	3	n/a	4	4	n/a	n/a	4	4	3	3	4	2	4	4	4	4	n/a	n/a	4

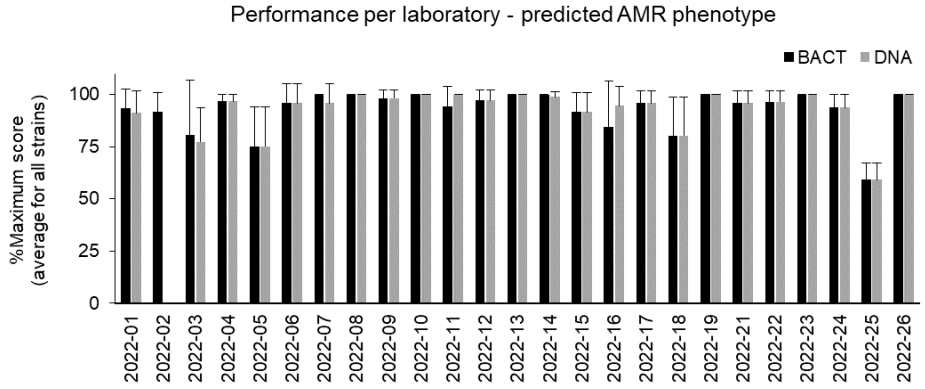

Figure 33. Performance of participants regarding the identification of expected predicted AMR phenotype (individual antimicrobials), expressed as percent of the maximum score. Average of all strains *and standard deviation are plotted.*

8. DATA ANALYSIS – MLST

The expected MLST type and allele values are presented in Table 20 (S. aureus), Table 21 (E. coli), Table 22 (E. faecium) and Table 23 (E. faecalis). The submitted data on MLST for all strains are presented in Figure 34. All participants identified the expected MLST for S. aureus, and all except for one participant for E. coli. There was a lot of confusion about the MLST analysis of E. faecium strain GENOMIC22-005, as it was not possible to determine the MLST due to lack of a hit for allel pstS in the pubmlst database. Finally, three participants did not identify an MLST type for strain GENOMIC22-006 (2022-15, 2022-16 and 2022-17).

Table 20. Expected sequence type and allele values for S. aureus.

Gene	Allele values – *S. aureus*	
	GENOMIC22-001	GENOMIC22-002
arcC	3	3
aroE	35	35
glpF	19	19
gmk	2	2
pta	20	20
tpi	26	26
yqiL	39	39
ST	**398**	**398**

Table 21. Expected sequence type and allele values for E. coli.

Gene	Allele values - *E. coli*	
	GENOMIC22-003	GENOMIC22-004
adk	35	6
fumC	37	4
gyrB	29	12
icd	25	1
mdh	4	20
purA	5	18
recA	73	7
ST	**405**	**410**

Table 22 E Expected sequence type and allele values for E. faecium.

Gene	Allele values – *E. faecium* GENOMIC22-005
adk	1
atpA	9
ddl	1
gdh	1
gyd	12
pstS	0
purK	1
ST	**0**

Table 23. Expected sequence type and allele values for E. faecalis.

Gene	Allele values – *E. faecalis* GENOMIC22-006
aroE	6
gdh	12
gki	7
gyd	7
pstS	3
xpt	1
yqiL	5
ST	**6**

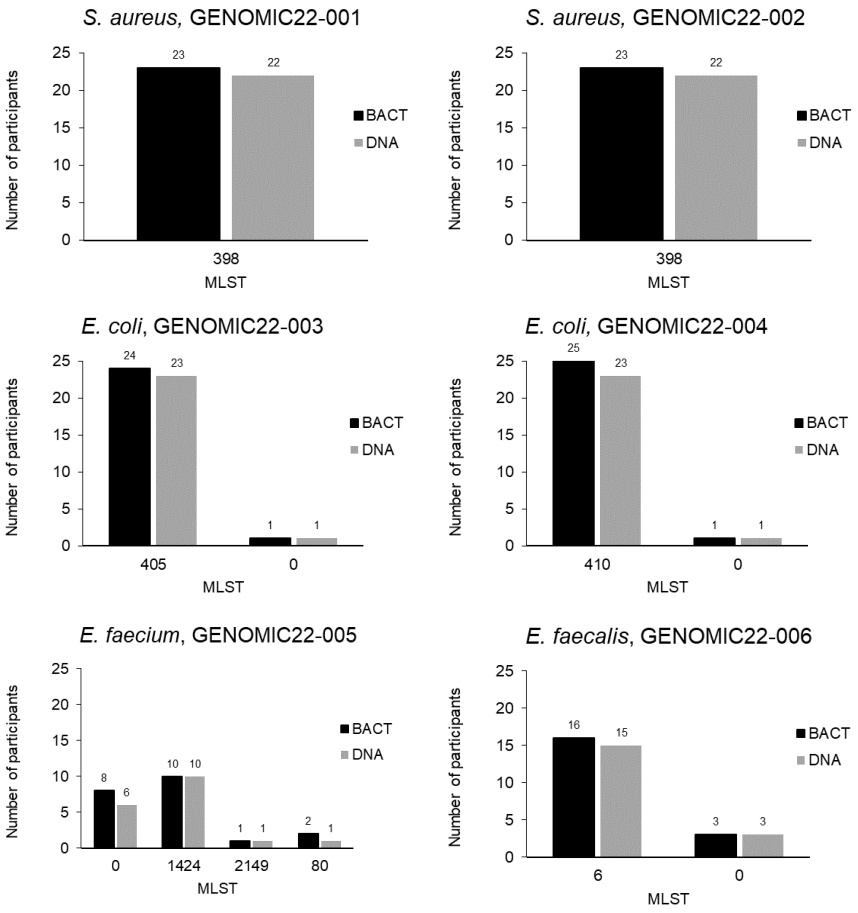

Figure 34. Submitted sequence types for all strains.