DTU Genomic Proficiency Test 2024 Report:

Interpretation of Submitted Data and Scoring System for *Escherichia coli*, *Staphylococcus aureus* and *Enterococcus* spp.

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1. INTRODUCTION

The DTU Genomic Proficiency Test (https://www.food.dtu.dk) is organized and provided by the Research Group on Global Capacity Building at the Technical University of Denmark (DTU) to support global harmonization and standardization in Whole Genome Sequencing (WGS) and bioinformatics analysis. The main objective is to enable laboratories worldwide to generate comparable, high-quality genomic data for surveillance and research purposes.

This document provides guidance for participants of the DTU Genomic Proficiency Test (PT) 2024, including members of the EURL-AMR, SEQAFRICA, and EQAsia networks. Its primary aim is to support the interpretation of the participants' individual evaluation reports and explain the scoring system applied to submitted results.

1.1. Scope

The DTU Genomic PT is a tool for performance evaluation of bioinformatics analyses employed in AMR surveillance. It enables participating laboratories to assess their ability to accurately identify and report the following elements from Whole Genome Sequencing (WGS) data:

- Antimicrobial resistance (AMR) genes
- AMR-associated mutations
- *In silico* prediction of AMR phenotypes
- Multilocus sequence types (MLST)
- Plasmid replicons

The strains included in the DTU Genomic PT 2024 are listed in **Table 1**, and the antimicrobial compounds evaluated for each organism are presented in **Table 2**. The expected results are limited to the organism-specific antimicrobial panels defined in **Table 2**. The antimicrobial compounds included for *E. coli*, *E. faecalis* and *E. faecium* follow the antimicrobials specified in the EU legislation on the monitoring and reporting of antimicrobial resistance in zoonotic and commensal bacteria (1). For *S. aureus* the selection is based on the EU legislation on the monitoring of methicillin-resistant *S. aureus* in fattening pigs (2).

According to the EUCAST guidelines "Expected Resistant Phenotypes, Version 1.2 January 2023", *E. faecalis* and *E. faecium* are expected to exhibit low level resistance to aminoglycosides and resitance to macrolides. Moreover, *E. faecalis* is considered to be resistant to quinupristin-dalfopristin. For such cases, EUCAST recommends that laboratories either refrain from reporting a result or to report the strain as resistant. As shown in **Table 2**, the antimicrobial panel for *E. faecalis* and *E. faecium* includes one aminoglycoside (gentamicin) and one macrolide (erythromycin). Results corresponding to expected resistant phenotypes are excluded from the analysis (see section 1.5, Scoring Categories).

Table 1. Strains included in the DTU Genomic PT 2024

Species	Strain code
Escherichia coli	GPT24-01
	GPT24-02
Staphylococcus aureus	GPT24-03
	GPT24-04
Enterococcus faecalis	GPT24-05
Enterococcus faecium	GPT24-06

Table 2. Antimicrobial compounds included in the DTU Genomic PT 2024. The antimicrobial panels for *E. coli*, *E. faecalis*, and *E. faecium* are based on EU legislation for the monitoring and reporting of antimicrobial resistance in zoonotic and commensal bacteria (1), while the panel for *S. aureus* is based on EU legislation for the monitoring of methicillin-resistant *S. aureus* in fattening pigs (2). ✓ = included; – = not included.

ANTIMICROBIAL CLASS	ANTIMICROBIAL COMPOUND	*E. coli*	*S. aureus*	*E. faecalis*/ *E. faecium*
AMINOGLYCOSIDES	Amikacin	√	–	–
	Gentamicin	√	√	√*
	Kanamycin	–	√	–
	Streptomycin	–	√	–
BETA-LACTAMS	Ampicillin	√	–	√
	Cefepime	√	–	–
	Cefotaxime	√	–	–
	Cefoxitin	√	√	–
	Ceftazidime	√	–	–
	Ertapenem	√	–	–
	Imipenem	√	–	–
	Meropenem	√	–	–
	Penicillin	–	√	–
	Temocillin	√	–	–
GLYCOPEPTIDES	Teicoplanin	–	–	√
	Vancomycin	–	√	√
LINCOSAMIDES	Clindamycin	–	√	–
LIPOPEPTIDES	Daptomycin	–	–	√
MACROLIDES	Azithromycin	√	–	–
	Erythromycin	–	√	√*
OXAZOLIDINONES	Linezolid	–	√	√
PHENICOLS	Chloramphenicol	√	√	√
PLEUROMUTILINS	Tiamulin	–	√	–
POLYMYXINS	Colistin	√	–	–
PSEUDOMONIC ACIDS	Mupirocin	–	√	–
QUINOLONES	Ciprofloxacin	√	√	√
	Nalidixic acid	√	–	–
RIFAMYCINS	Rifampin	–	√	–
STEROID ANTIBACTERIALS	Fusidic acid	–	√	–
STREPTOGRAMINS	Quinupristin-dalfopristin	–	√	√*
SULFONAMIDES	Sulfamethoxazole	√	√	–
TETRACYCLINES	Tetracycline	√	√	√
	Tigecycline	√	–	√
TRIMETHOPRIM	Trimethoprim	√	√	–

*According to EUCAST guidelines "Expected Resistant Phenotypes, Version 1.2 January 2023", the expected resistant phenotypes include low level resistance to aminoglycosides (gentamicin) and resistance to macrolides (erythromycin) in *E. faecalis* and *E. faecium*, as well as resistance to quinupristin-dalfopristin for *E. faecalis*. For details, see the "Scope" section.

1.2. Expected Data Generation

Expected results were generated using high-quality hybrid genome assemblies of the reference strains, which served as the ground truth for evaluation. The closed genomes of the reference strains were obtained using the pipeline described below.

1.2.1. LONG READ SEQUENCING:

- Platform: Oxford Nanopore Technology (ONT) MinION Mk1B
- Flowcell: R10.4
- Library Preparation: SQK-NBD114-24
- Basecalling: Dorado (version 7.8.3)
- Quality Control and Trimming: Nanoplot (version 1.44.1), Chopper (version 0.9.1), Porechop (version 0.2.4), and Filtlong (version 0.2.1) with the following parameters "--min_length 1000 --keep_percent 90 --trim --split 500" and using Illumina raw reads as reference

1.2.2. SHORT READ SEQUENCING:

- Platform: Illumina NextSeq 500 (Paired-end 2x150 bp)
- Library Preparation: Nextera XT Kit
- Quality Control and Trimming: FastQC (version 0.12.1), MultiQC (version 1.23) and Fastp (version 0.23.4)

1.2.3. CONTAMINATION ASSESSMENT:

- Species determination and contamination were assessed using Kraken2 (version 2.1.3), Bracken (version 2.9), and Krona (version 2.8.1). Intraspecies contamination was evaluated using ConFindr (version 0.8.2) and the rMLST databases from PubMLST.

1.2.4. HYBRID ASSEMBLY:

- A first draft assembly was generated using Autocycler (version 0.4.0) using ONT reads.
- The dnaapler (version 1.2.0) was used for reorienting the complete bacterial genome assemblies using the GFA output file from Autocycler.
- Polishing was performed with Polypolish (version 0.6.0) using Illumina short reads.
- A second round of polishing was done with Pypolca (version 0.3.1) but the changed regions were manually inspected.
- To ensure comprehensive plasmid recovery, Unicycler (version 0.5.1) was also used to confirm the presence of very small plasmids.

1.2.5. QUALITY CONTROL OF THE ASSEMBLED GENOMES:

- Quality control of assembled genomes was carried out using QUAST (version 5.3.0), completeness and contamination were evaluated using CheckM2 (version 1.1.0), and BUSCO (version 5.8.3).

1.2.6. GENOME ANNOTATION:

- Annotation was performed using the **Rapid Annotation using Subsystem Technology** (RAST) toolkit. The tools, software versions, databases, and thresholds used to generate expected results are provided in **Table 3**.

Table 3. Tools used for the prediction of the expected results from the reference genomes.

Type of analysis	Tool Name, platform	Tool Version	Database Version	Thresholds Used (COV, coverage; ID, Identity)	Reference
MLST	MLST 2.0.9, CGE	2022-05-11	2023-06-19	100% ID, 100% COV	(3)
AMR	ResFinder, CGE	2024-03-22	ResFinder: (2024-03-22) PointFinder: (2024-03-08)	95% ID, 95% COV	(4)
	NCBI AMRFinderPlus, command line	4.0.23	v2025-03-25.1	95% ID, 95% COV	(5)
Plasmid typing	PlasmidFinder, CGE	2.0.1, 2020-07-01	2023-01-18	95% ID, 90% COV	(6)
	MOB-suite, Galaxy	3.0.3	N/A	95% ID, 90% COV	(7)

1.3. Submission of genomic data to the European Nucleotide Archive (ENA)

WGS raw data from Illumina and ONT (MinION) sequencing platforms, as well as the hybrid assemblies, were submitted to the European Nucleotide Archive (ENA) (please refer to the individual accession numbers for each Genomic PT (GPT) reference strain provided in **Table 4** below).

Table 4. Accession numbers of genomic data from the DTU Genomic PT 2024 reference strains submitted to ENA.

Organism	Strain	Study Accession	Sample Accession	Accession Number
Escherichia coli	GPT24-01	PRJEB86006	ERS23842151	GCA_965641705
	GPT24-02	PRJEB86006	ERS23842152	GCA_965641715
Staphylococcus aureus	GPT24-03	PRJEB86006	ERS23842153	GCA_965641745
	GPT24-04	PRJEB86006	ERS23842154	GCA_965641735
Enterococcus faecalis	GPT24-05	PRJEB86006	ERS23842155	GCA_965641725
Enterococcus faecium	GPT24-06	PRJEB86006	ERS23842156	GCA_965641755

1.4. Genome assembly summary and plasmid content

All six bacterial strains included in the DTU Genomic PT 2024 were fully closed, comprising six chromosomes and nineteen plasmids (**Table 5**).

Table 5. Information on the closed genome for each strain of the DTU Genomic PT 2024.

Organism	Strain	Molecule type	Size (bp)	GC%
E. coli	GPT24-01	Chromosome	5,229,427	50.6
		Plasmid_1	132,041	50.7
		Plasmid_2	96,237	47.6
		Plasmid_3	32,937	41.4
		Plasmid_4	2,101	47.2
		Plasmid_5	1,549	51.1

Table 5. Information on the closed genome for each strain of the DTU Genomic PT 2024.				
Organism	**Strain**	**Molecule type**	**Size (bp)**	**GC%**
	GPT24-02	Chromosome	5,159,857	50.7
		Plasmid_1	130,631	48.6
		Plasmid_2	102,589	50.8
		Plasmid_3	46,125	44.3
		Plasmid_4	33,303	41.9
		Plasmid_5	7,176	45.6
		Plasmid_6	4,779	51.4
		Plasmid_7	4,315	43.7
		Plasmid_8	1,541	51.7
S. aureus	GPT24-03	Chromosome	2,783,198	32.9
		Plasmid_1	3,710	34.8
	GPT24-04	Chromosome	2,819,759	32.9
		Plasmid_1	54,220	29.1
E. faecalis	GPT24-05	Chromosome	2,825,666	37.6
		Plasmid_1	70,177	34.7
		Plasmid_2	36,331	34.4
E. faecium	GPT24-06	Chromosome	2,777,239	38.5
		Plasmid_1	208,043	35.4
		Plasmid_2	10,366	31.1

1.5. Scoring Categories

Submitted results are classified into predefined scoring categories to ensure consistent and transparent evaluation across all participants. Each scoring category is described in **Table 6** below:

Table 6. Scoring categories used for the evaluation of submitted genomic results in the DTU Genomic PT 2024. Each category defines how submitted results are classified and scored based on their correspondence with the reference genome, expected dataset, or reporting format.

Scoring Category (Abbreviation)	Description	Score if submitted	Score if not submitted
Expected result (EXP)	Submitted result **is present** in the reference genome and is part of the expected results.	**1**	**0**
Not expected result (N-EXP)	Submitted result is **not present** in the reference genome and is not part of the expected results.	**0**	**-**
Accepted (ACC)	Submitted result is found on the reference genome but is not part of the predefined expected dataset, due to specific reasons. For example, an AMR gene may be identified by one AMR prediction tool (e.g., AMRFinderPlus) but not by others (e.g., ResFinder), depending on tool-specific parameters. These cases are explained individually in this guide.	**1**	**-**
Out of scope (OOS)	Submitted result is present in the reference genome but is outside the defined scope of the GPT evaluation (e.g. detection of resistance to antimicrobials not included in the panel). These results are acknowledged for completeness but are excluded from scoring, whether submitted or not.	**-**	**-**

Table 6. Scoring categories used for the evaluation of submitted genomic results in the DTU Genomic PT 2024. Each category defines how submitted results are classified and scored based on their correspondence with the reference genome, expected dataset, or reporting format.

Scoring Category (Abbreviation)	Description	Score if submitted	Score if not submitted
Not acquired resistance (NAR)	Submitted result is part of the Expected Resistant phenotypes in a given species (see EUCAST rules). Laboratories are expected to either not report a result at all, or if a result is desired, to report the isolate as resistant. These results are acknowledged for completeness but are excluded from scoring, whether submitted or not.	-	-
Format issue (FORM)	Submitted result is correct in content but does not adhere to the required reporting format (e.g., incorrect mutation syntax).	1	-

1.6. How to Use This Document

Each of the following sections corresponds to one of the strains included in the DTU Genomic PT 2024. For each strain, the sections provide:

- The **expected results** for MLST, antimicrobial resistance (AMR) genes, AMR-associated mutations, phenotypic AMR profiles, and plasmid replicons.
- The **evaluation of participants' submitted results**, including comments, scoring categories (as defined in Table 6), and justifications for deviations or accepted variations.

This structure allows users to compare their submitted data against the reference results and understand the rationale behind the assigned scores. An overview of all tables included in the document is provided below. This overview serves as a navigation guide to help readers locate the relevant data and evaluations for each organism included in the DTU Genomic PT 2024.

DOCUMENT NAVIGATION GUIDE		
Organism / Strain	**Table No.**	**Content Description**
All strains	7	Expected MLST data
	8	Evaluation of submitted MLST data
***E. coli* GPT24-01**	9–11	• Expected AMR results • Submitted AMR results • Scoring and rationale
***E. coli* GPT24-02**	12–14	
***S. aureus* GPT24-03**	15–17	
***S. aureus* GPT24-04**	18–20	
***E. faecalis* GPT24-05**	21–23	
***E. faecium* GPT24-06**	24–26	

2. MULTILOCUS-SEQUENCE TYPING (MLST)

2.1. MLST Expected Data – All Strains

Table 7. Expected MLST data.									
Organism	Strain	Sequence Type	Allele 1	Allele 2	Allele 3	Allele 4	Allele 5	Allele 6	Allele 7
E. coli	GPT24-01	**405**	*adk*_35	*fumC*_37	*gyrB*_29	*icd*_25	*mdh*_4	*purA*_5	*recA*_73
	GPT24-02	**5759**	*adk*_507	*fumC*_93	*gyrB*_406	*icd*_594	*mdh*_365	*purA*_2	*recA*_2
S. aureus	GPT24-03	**130**	*arcC*_6	*aroE*_57	*glpF*_45	*gmk*_2	*pta*_7	*tpi*_58	*yqiL*_52
	GPT24-04	**15**	*arcC*_13	*aroE*_13	*glpF*_1	*gmk*_1	*pta*_12	*tpi*_11	*yqiL*_13
E. faecalis	GPT24-05	**22**	*aroE*_1	*gdh*_1	*gki*_1	*gyd*_7	*pstS*_10	*xpt*_10	*yqiL*_1
E. faecium	GPT24-06	**17**	*adk*_1	*atpA*_1	*ddl*_1	*gdh*_1	*gyd*_1	*pstS*_1	*purK*_1

2.2. MLST Submitted Data – All Strains

Table 8. Evaluation of the submitted MLST data.						
Organism	Strain	Submitted Sequence Type	No of labs	Score if submitted	Score if not submitted	Comment
E. coli	GPT24-01	**405**	34	1	0	EXP
	GPT24-02	**5759**	35	1	0	EXP
S. aureus	GPT24-03	**130**	33	1	0	EXP
	GPT24-04	**15**	33	1	0	EXP
		0	1	0	-	N-EXP
E. faecalis	GPT24-05	**22**	30	1	0	EXP
E. faecium	GPT24-06	**17**	30	1	0	EXP

3. ANTIMICROBIAL RESISTANCE (AMR) DATA

3.1. Expected AMR data for *E. coli* strain GPT24-01

Table 9 - Expected results for *E. coli* strain GPT24-01. Predicted resistant phenotypes for specific antimicrobials are indicated in **bold**. Percent identity and coverage were calculated at protein level.

Class (A-Z)	Antimicrobial	Predicted AMR Phenotype	AMR Gene	AMR Mutation	Protein name	%Identity	%Coverage
AMINOGLYCOSIDES	Amikacin	Susceptible	None detected	-	-	-	-
	Gentamicin						
BETA-LACTAMS	**Ampicillin**	**Resistant**	***bla*$_{CTX-M-15}$**	**-**	**Extended-spectrum class A beta-lactamase CTX-M-15**	**100.0**	**100.0**
			***bla*$_{NDM-5}$**	**-**	**Subclass B1 metallo-beta-lactamase NDM-5**	**100.0**	**100.0**
			***bla*$_{TEM-1}$**	**-**	**Broad-spectrum class A beta-lactamase TEM-1**	**100.0**	**100.0**
	• Cefepime • Cefotaxime • Ceftazidime	**Resistant**	***bla*$_{CTX-M-15}$**	**-**	**Extended-spectrum class A beta-lactamase CTX-M-15**	**100.0**	**100.0**
			***bla*$_{NDM-5}$**	**-**	**Subclass B1 metallo-beta-lactamase NDM-5**	**100.0**	**100.0**
	• Cefoxitin • Ertapenem • Imipenem • Meropenem • Temocillin	**Resistant**	***bla*$_{NDM-5}$**	**-**	**Subclass B1 metallo-beta-lactamase NDM-5**	**100.0**	**100.0**
MACROLIDES	**Azithromycin**	**Resistant**	***mph(A)***	**-**	**Mph(A) family macrolide 2'-phosphotransferase**	**100.0**	**100.0**
PHENICOLS	Chloramphenicol	Susceptible	None detected	-	-	-	-
POLYMYXINS	Colistin	Susceptible	None detected	-	-	-	-
QUINOLONES	**Ciprofloxacin**	**Resistant**	***gyrA***	**D87N S83L**	***Escherichia* quinolone resistant GyrA**	**98.9**	**99.7**
			parC	**S80I**	***Escherichia* quinolone resistant ParC**	**99.9**	**100.0**
			parE	**S458A**	***Escherichia* quinolone resistant ParE**	**99.7**	**100.0**
			qepA4	**-**	**Fluoroquinolone efflux MFS transporter QepA4**	**100.0**	**100.0**

Table 9 - Expected results for *E. coli* strain GPT24-01. Predicted resistant phenotypes for specific antimicrobials are indicated in **bold**. Percent identity and coverage were calculated at protein level.

Class (A-Z)	Antimicrobial	Predicted AMR Phenotype	AMR Gene	AMR Mutation	Protein name	%Identity	%Coverage
	Nalidixic acid	**Resistant**	***gyrA***	**D87N, S83L**	**Escherichia quinolone resistant GyrA**	**98.9**	**99.7**
			parC	**S80I**	***Escherichia* quinolone resistant ParC**	**99.9**	**100.0**
			parE	**S458A**	***Escherichia* quinolone resistant ParE**	**99.7**	**100.0**
SULFONAMIDES	**Sulfamethoxazole**	**Resistant**	***sul1***	**-**	**Sulfonamide-resistant dihydropteroate synthase Sul1**	**100.0**	**100.0**
TETRACYCLINES	**Tetracycline**	**Resistant**	***tet(B)***	**-**	**Tetracycline efflux MFS transporter Tet(B)**	**100.0**	**100.0**
	Tigecycline	Susceptible	None detected	-	-	-	-
TRIMETHOPRIM	**Trimethoprim**	**Resistant**	***dfrA12***	**-**	**Trimethoprim-resistant dihydrofolate reductase DfrA12**	**100.0**	**100.0**

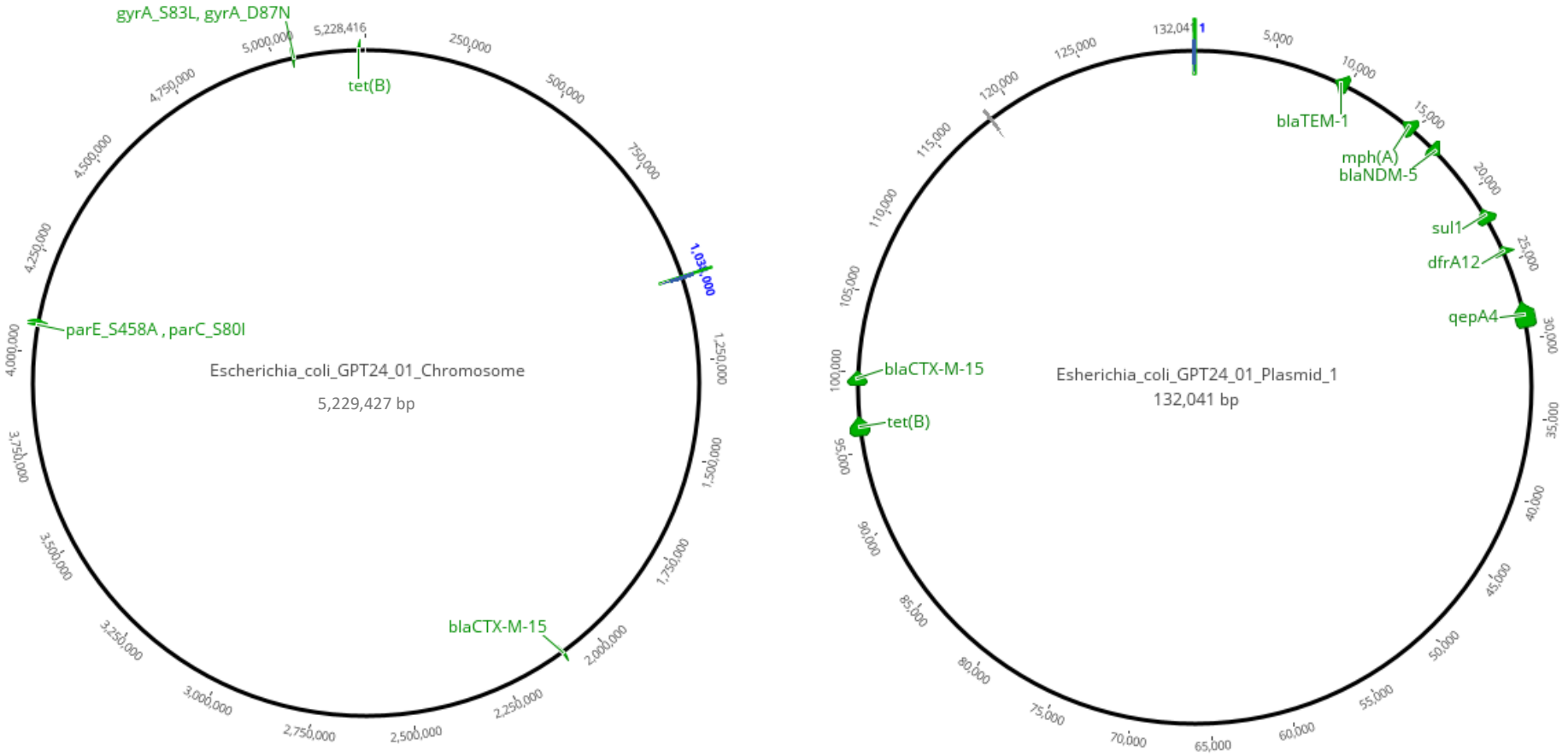

Figure 1. *Location of AMR genes or genes with mutations associated to AMR in E. coli strain GPT24-01.* ***Left****: Chromosome,* ***Right****: Plasmid_1.*

3.2. Submitted AMR genes and mutations for *E. coli* strain GPT24-01

Table 10. Submitted AMR Genes and Mutations for *E. coli* strain GPT24-01.

Class (A-Z)	AMR Gene	AMR mutation	No of Laboratories	Score if submitted	Score if not submitted	Scoring Category
AMINOGLYCOSIDES	*aadA2*	-	7	-	-	OOS[1]
	ant(3")-Ia	-	1	-	-	OOS[1]
	aadA8b	-	1	0	-	N-EXP
	aph(3')-III	-	1	0	-	N-EXP
	aph(3')-IIIa	-	1	0	-	N-EXP
BETA-LACTAMS	$bla_{CTX-M-15}$	-	33	1	0	EXP
	bla_{NDM-5}	-	33	1	0	EXP
	bla_{TEM-1}	-	11	1	0	EXP[2]
	bla_{TEM-1B}	-	20			
	blaEC	-	2	-	-	NAR[3]
MACROLIDES	*mph(A)*	-	32	1	0	EXP
	23S	1229C>T	1	0	-	N-EXP
		1483G>A	1	0	-	N-EXP
		1723G>A	1	0	-	N-EXP
		1725T>C	1	0	-	N-EXP
		1726C>G	1	0	-	N-EXP
		1727C>A	1	0	-	N-EXP
		1730C>T	1	0	-	N-EXP
		1733G>T	1	0	-	N-EXP
		1734G>C	1	0	-	N-EXP
		1735A>G	1	0	-	N-EXP
		1870C>G	1	0	-	N-EXP
		1871A>T	1	0	-	N-EXP
		1873_1874insAC	1	0	-	N-EXP
		2205A>G	1	0	-	N-EXP
		2211A>T	1	0	-	N-EXP
		2794C>T	1	0	-	N-EXP
		2796T>C	1	0	-	N-EXP
		2799A>G	1	0	-	N-EXP
		2802G>A	1	0	-	N-EXP
	erm(D)	-	1	0	-	N-EXP
	erm(E)	-	1	0	-	N-EXP
POLYMYXINS	*pmrA*	G144S	3	0	-	N-EXP
	pmrB	H2R,D283G	1	0	-	N-EXP
		D283G	2	0	-	N-EXP
		H2R	2	0	-	N-EXP
QUINOLONES	*gyrA*	S83L	21	1	0	EXP
		D87N	22	1	0	EXP

[1] The gene *aadA2* (also referred to as *ant(3")-Ia*), encoding the ANT(3")-Ia family aminoglycoside nucleotidyltransferase AadA2, is located on Plasmid_1 with 100% identity and coverage. Although this gene is associated with resistance to streptomycin, this antimicrobial is not included in the panel for *E. coli* (**Table 2**) and is therefore considered out of scope for this analysis.

[2] The gene may be reported as bla_{TEM-1} or bla_{TEM-1B}, depending on the antimicrobial resistance prediction tool applied.

[3] The *blaEC* is a chromosomal *ampC* gene which is constitutively expressed in *E. coli* at a low level, while it is not inducible in the presence of β-lactam antibiotics, like in other bacterial species (8–10). As such, it is considered naturally occurring and falls under the NAR category. While its detection is correct, it is excluded from scoring, as the gene is not relevant to the evaluation of acquired resistance, which is the focus of the DTU Genomic PT.

Table 10. Submitted AMR Genes and Mutations for *E. coli* strain GPT24-01.						
Class (A-Z)	**AMR Gene**	**AMR mutation**	**No of Laboratories**	**Score if submitted**	**Score if not submitted**	**Scoring Category**
		gyrA_p.D87N	1	1	-	FORM[4]
		gyrA_p.S83L	1	1	-	FORM[4]
		S83L[5]	1	1	-	FORM[4]
		gyrA_S83L	1	1	-	FORM[4]
		S83L,D87N	1	1	-	FORM[4]
		gyrAp.D87N	1	1	-	FORM[4]
		gyrAp.S83L	1	1	-	FORM[4]
		p.S83L,p.D87N	2	1	-	FORM[4]
		p.D87N	4	1	-	FORM[4]
		p.S83L	4	1	-	FORM[4]
		D678E	3	0	-	N-EXP
	gyrB	A618T,T653A,I663V	1	0	-	N-EXP
		T653A	2	0	-	N-EXP
		I663V	2	0	-	N-EXP
		A618T	2	0	-	N-EXP
	parC	S80I	24	1	0	EXP
		parCp.S80I	1	1	-	FORM[4]
		parC_p.S80I	1	1	-	FORM[4]
		parC_S801	1	1	-	FORM[4]
		p.S80I	6	1	-	FORM[4]
	parE	S458A	22	1	0	EXP
		parEp.S458A	1	1	-	FORM[4]
		parE_p.S458A	1	1	-	FORM[4]
		parE_S458A	1	1	-	FORM[4]
		p.S458A	6	1	-	FORM[4]
	qepA4	-	31	1	0	EXP
	qepA	-	1	1	-	ACC[6]
SULFONAMIDES	*sul1*	-	31	1	0	EXP
	sul2	-	1	0	-	N-EXP
	folP	F4Y	3	0	-	N-EXP
TETRACYCLINES	*tet(B)*	-	32	1	0	EXP
	tet	-	1	0	-	N-EXP
TRIMETHOPRIM	*dfrA12*	-	32	1	0	EXP
	dfrA8	-	1	0	-	N-EXP
	dfrG	-	1	0	-	N-EXP

[4] The DTU Genomic PT protocol specified that mutation submissions should be performed by first selecting the corresponding gene from a drop-down menu, followed by entering the mutation in a free-text field using a defined format for substitutions: original amino acid name using the amino acid one-letter code, position in the protein, and final amino acid (one-letter code) – *e.g.*, S83L. Some participants submitted the correct mutations but used a different format. As a result, the webtool did not recognize these entries as matching the expected data, which were validated against the mandatory format. This represents a limitation of the current version of the webtool. In the upcoming version of the webtool to be launched in the next DTU Genomic PT round, this issue will be addressed by replacing the free-text entry with a standardized drop-down menu listing all expected mutations, thereby eliminating format-related discrepancies. To ensure fairness, participant scores in cases affected by this issue will be corrected during the review phase of this guidance document, and the updated scores will replace those initially generated by the webtool in the webtool reports also.

[5] Space in the end led to mismatch with expected data. See footnote 4.

[6] The *qepA4* allele is a variant of the *qepA* gene, which encodes an efflux pump conferring decreased susceptibility to fluoroquinolones. Depending on the database and/or tool used (e.g., ResFinder, AMRFinderPlus), allele-level annotation may differ. Some tools report the specific variant (e.g., *qepA4*), while others report only the name of the gene (*qepA*). Both designations refer to the same resistance determinant and are therefore considered equivalent for interpretation and scoring.

3.3. Submitted AMR phenotype for *E. coli* strain GPT24-01

Table 11. Submitted predicted AMR phenotype for *E. coli* strain GPT24-01.

Class (A-Z)	Predicted Resistant Phenotype to Antimicrobial	No of Laboratories	Score if submitted	Score if not submitted	Scoring Category
AMINOGLYCOSIDES	Amikacin	1	**0**	-	N-EXP
BETA-LACTAMS	Ampicillin	32	**1**	**0**	EXP
	Cefepime	30	**1**	**0**	EXP
	Cefotaxime	31	**1**	**0**	EXP
	Cefoxitin	31	**1**	**0**	EXP
	Ceftazidime	31	**1**	**0**	EXP
	Ertapenem	30	**1**	**0**	EXP
	Imipenem	29	**1**	**0**	EXP
	Meropenem	31	**1**	**0**	EXP
	Temocillin	29	**1**	**0**	EXP
MACROLIDES	Azithromycin	30	**1**	**0**	EXP
QUINOLONES	Ciprofloxacin	31	**1**	**0**	EXP
	Nalidixic acid	26	**1**	**0**	EXP
SULFONAMIDES	Sulfamethoxazole	32	**1**	**0**	EXP
TETRACYCLINES	Tetracycline	33	**1**	**0**	EXP
TRIMETHOPRIM	Trimethoprim	31	**1**	**0**	EXP

3.4. Expected AMR data for *E. coli* strain GPT24-02

Table 12- Expected results for *E. coli* strain GPT24-02. Predicted resistant phenotypes for specific antimicrobials are indicated in **bold**. Percent identity and coverage were calculated at protein level.

Class (A-Z)	Antimicrobial	Predicted AMR Phenotype	AMR Gene	AMR Mutation	Protein name	%Identity	%Coverage
AMINOGLYCOSIDES	Amikacin	Susceptible	None detected	-	-	-	-
	Gentamicin						
BETA-LACTAMS	**Ampicillin**	**Resistant**	***bla*$_{TEM-1}$**	**-**	**Broad-spectrum class A beta-lactamase TEM-1**	**100.0**	**100.0**
	• Cefepime • Cefotaxime • Cefoxitin • Ceftazidime • Ertapenem • Imipenem • Meropenem • Temocillin	Susceptible	None detected	-	-	-	-
MACROLIDES	Azithromycin	Susceptible	None detected	-	-	-	-
PHENICOLS	Chloramphenicol	Susceptible	None detected	-	-	-	-
POLYMYXINS	**Colistin**	**Resistant**	***mcr-1.1***	**-**	**Phosphoethanolamine--lipid A transferase MCR-1.1**	**100.0**	**100.0**
QUINOLONES	• Ciprofloxacin • Nalidixic acid	Susceptible	None detected	-	-	-	-
SULFONAMIDES	Sulfamethoxazole	Susceptible	None detected	-	-	-	-
TETRACYCLINES	Tetracycline	Susceptible	None detected	-	-	-	-
TRIMETHOPRIM	Trimethoprim	Susceptible	None detected	-	-	-	-

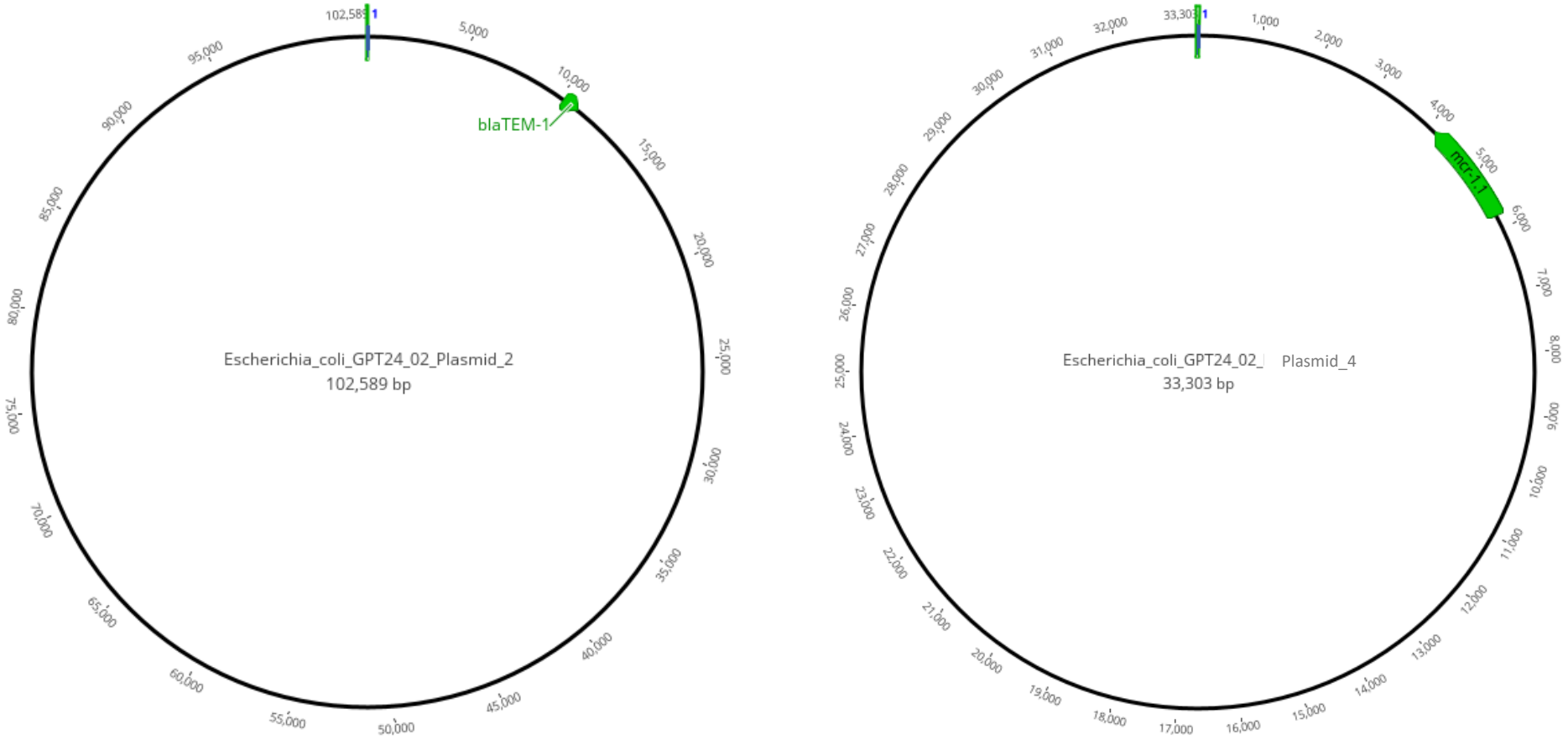

***Figure 2**. Location of AMR genes or genes with mutations associated to AMR in E. coli strain GPT24-02. **Left**: Plasmid 2, **Right**: Plasmid 3.*

3.5. Submitted AMR genes and mutations for *E. coli* strain GPT24-02

Table 13. Submitted AMR genes for *E. coli* strain GPT24-02.						
Class (A-Z)	**AMR Gene**	**AMR mutation**	**No of Laboratories**	**Score if submitted**	**Score if not submitted**	**Scoring Category**
AMINOGLYCOSIDES	*aph(3')-Ib*	-	1	**0**	-	N-EXP
	aph(6)-Id	-	9	-	-	OOS[7]
	aph(3")-Ib	-	7	-	-	OOS[7]
	16S-rrsH	r.1120T>C	1	**0**	-	N-EXP
		1120T>C	1	**0**	-	N-EXP
BETA-LACTAMS	bla_{TEM-1C}	-	25	**1**	**0**	EXP
	bla_{TEM-1}	-	9			
	blaZ	-	1	**0**	-	N-EXP
	mecC	-	1	**0**	-	N-EXP
	blaEC	-	2	-	-	NAR[3]
MACROLIDES	*23S*	356G>A	1	**0**	-	N-EXP
		264C>G	1	**0**	-	N-EXP
		1211C>T	1	**0**	-	N-EXP
		1595C>T	1	**0**	-	N-EXP
		316C>T	1	**0**	-	N-EXP
		1723G>A	1	**0**	-	N-EXP
		547A>C	1	**0**	-	N-EXP
		1865T>C	1	**0**	-	N-EXP
		2132T>A	1	**0**	-	N-EXP
POLYMYXINS	*mcr-1.1*	-	33	**1**	**0**	EXP
	pmrB	V351I	2	**0**	-	N-EXP
		p.V351I	1	**0**	-	N-EXP
		H2R	2	**0**	-	N-EXP
		p.H2R	1	**0**	-	N-EXP
QUINOLONES	*gyrA*	p.D678E	1	**0**	-	N-EXP
		D678E	2	**0**	-	N-EXP

3.6. Predicted AMR phenotype for *E. coli* strain GPT24-02

Table 14. Submitted predicted AMR phenotype for *E. coli* strain GPT24-02.					
Class (A-Z)	**Predicted Resistant Phenotype to Antimicrobial**	**No of Laboratories**	**Score if submitted**	**Score if not submitted**	**Scoring Category**
BETA-LACTAMS	Ampicillin	32	**1**	**0**	EXP
POLYMYXINS	Colistin	33	**1**	**0**	EXP

[7] The genes *aph(6)-Id* and *aph(3")-Ib*, both encoding aminoglycoside O-phosphotransferases that are predicted to confer resistance to streptomycin, are located on Plasmid_2 with 100% identity and coverage. As streptomycin is not included in the antimicrobial panel for *E. coli* (**Table 2**), these genes are considered out of scope (OOS) for this analysis.

3.7. Expected AMR data for *S. aureus* strain GPT24-03

Table 15. Expected results for *S. aureus* strain GPT24-03. Predicted resistant phenotypes for specific antimicrobials are indicated in **bold**.
Percent identity and coverage were calculated at protein level.

Class (A-Z)	Antimicrobial	Predicted AMR Phenotype	AMR Gene	AMR Mutation	Protein name	%Identity	%Coverage
AMINOGLYCOSIDES	Gentamicin	Susceptible	None detected	-	-	-	-
	Kanamycin						
	Streptomycin						
BETA-LACTAMS	**Cefoxitin**	**Resistant**	***mecC***	**-**	**PBP2a family beta-lactam-resistant peptidoglycan transpeptidase MecC**	**100.0**	**100.0**
	Penicillin	**Resistant**	***mecC***	**-**	**PBP2a family beta-lactam-resistant peptidoglycan transpeptidase MecC**	**100.0**	**100.0**
			blaZ	**-**	**MecC-type penicillin-hydrolyzing class A beta-lactamase BlaZ**	**100.0**	**100.0**
GLYCOPEPTIDES	Vancomycin	Susceptible	None detected	-	-	-	-
LINCOSAMIDES	Clindamycin	Susceptible	None detected	-	-	-	-
MACROLIDES	Erythromycin	Susceptible	None detected	-	-	-	-
OXAZOLIDINONES	Linezolid	Susceptible	None detected	-	-	-	-
PHENICOLS	Chloramphenicol	Susceptible	None detected	-	-	-	-
PLEUROMUTILINS	Tiamulin	Susceptible	None detected	-	-	-	-
PSEUDOMONIC ACIDS	Mupirocin	Susceptible	None detected	-	-	-	-
QUINOLONES	Ciprofloxacin	Susceptible	None detected	-	-	-	-
RIFAMYCINS	Rifampin	Susceptible	None detected	-	-	-	-
STEROID ANTIBACTERIALS	Fusidic acid	Susceptible	None detected	-	-	-	-
STREPTOGRAMINS	Quinupristin/ dalfopristin	Susceptible	None detected	-	-	-	-
SULFONAMIDES	Sulfamethoxazole	Susceptible	None detected	-	-	-	-
TETRACYCLINES	Tetracycline	Susceptible	None detected	-	-	-	-
TRIMETHOPRIM	Trimethoprim	Susceptible	None detected	-	-	-	-

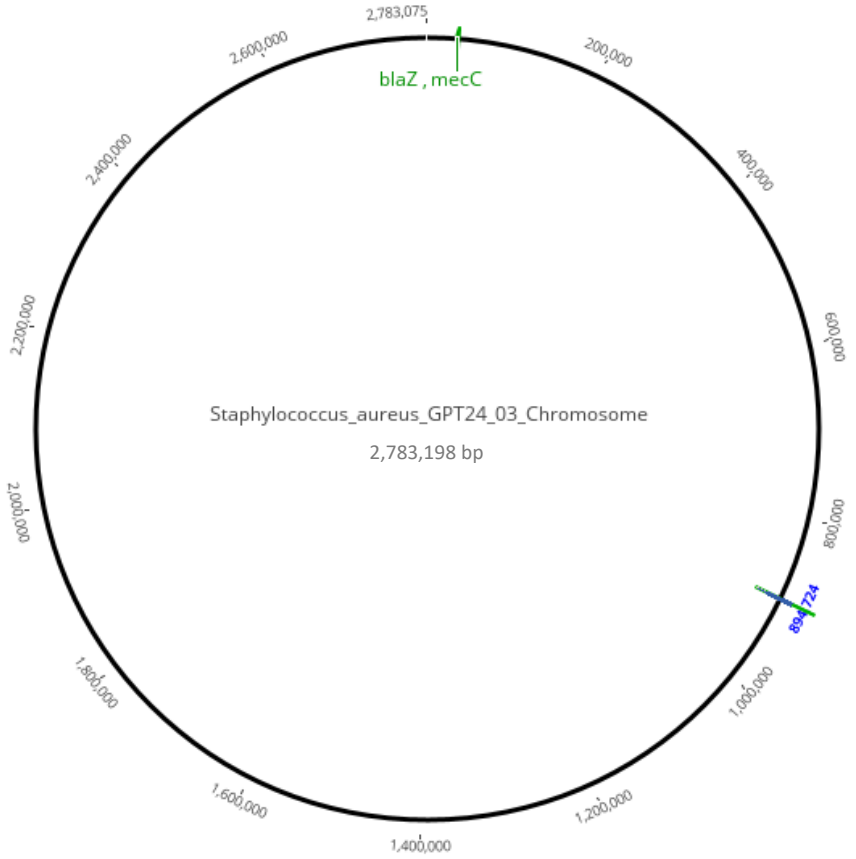

***Figure 3**. Location of AMR genes or genes with mutations associated to AMR in S. aureus strain GPT24-03 chromosome.*

3.8. Submitted AMR genes and mutations for *S. aureus* strain GPT24-03

Table 16 - Submitted AMR genes for *S. aureus* strain GPT24-03.						
Class (A-Z)	**AMR Gene**	**AMR mutation**	**No of Laboratories**	**Score if submitted**	**Score if not submitted**	**Scoring Category**
BETA-LACTAMS	*mecC*	-	33	1	0	EXP
	blaZ	-	31	1	0	EXP
	pbp2	p.T439V	1	0	-	N-EXP
		p.A606D	1	0	-	N-EXP
		A576S	1	0	-	N-EXP
		A606D	1	0	-	N-EXP
		p.E315A	1	0	-	N-EXP
		E315A	1	0	-	N-EXP
		T439V	1	0	-	N-EXP
		E315A,T439V, A576S,A606D	1	0	-	N-EXP
		p.A576S	1	0	-	N-EXP
	pbp4	p.T409A	1	0	-	N-EXP
		E398A	1	0	-	N-EXP
		T25A,L234H,K349E, E398A,T409A (Promoter: n.-54A>G, n.-160A>G, p.T25A)	1	0	-	N-EXP
		K349E	1	0	-	N-EXP
		p.T25A	1	0	-	N-EXP
		L234H	1	0	-	N-EXP
		T25A	1	0	-	N-EXP
		p.E398A	1	0	-	N-EXP
		T409A	1	0	-	N-EXP
		p.K349E	1	0	-	N-EXP
		p.L234H	1	0	-	N-EXP
PSEUDOMONIC ACIDS	*ileS*	p.D172E	1	0	-	N-EXP
		p.K286N	1	0	-	N-EXP
		p.E259D	1	0	-	N-EXP
		D172E,N213D,E259D, K286N,D425E,H510N	1	0	-	N-EXP
		D172E	1	0	-	N-EXP
		D425E	1	0	-	N-EXP
		p.D425E	1	0	-	N-EXP
		E259D	1	0	-	N-EXP
		p.H510N	1	0	-	N-EXP
		H510N	1	0	-	N-EXP
		p.N213D	1	0	-	N-EXP
		K286N	1	0	-	N-EXP
		N213D	1	0	-	N-EXP
QUINOLONES	*grlA/parC*	G697D	2	0	-	N-EXP
		V694M	1	0	-	N-EXP
		V694M,G697D	1	0	-	N-EXP
		p.G697D	1	0	-	N-EXP
		p.V694M	1	0	-	N-EXP
	grlB	E422D	3	0	-	N-EXP
		D530G	1	0	-	N-EXP
		p.E422D	1	0	-	N-EXP

Table 16 - Submitted AMR genes for *S. aureus* strain GPT24-03.						
Class (A-Z)	**AMR Gene**	**AMR mutation**	**No of Laboratories**	**Score if submitted**	**Score if not submitted**	**Scoring Category**
		p.E422D	1	0	-	N-EXP
		p.E422D,gaa>gat,e->d,	1	0	-	N-EXP
		E422D,D530G	1	0	-	N-EXP
		p.D530	1	0	-	N-EXP
	gyrA	T818_N819delinsN	2	0	-	N-EXP
		T825_S826del	1	0	-	N-EXP
		E887D	1	0	-	N-EXP
		E817V	1	0	-	N-EXP
		D483E,E815D,D816E, E817V,T818del,825_S826del	1	0	-	N-EXP
		D816E	1	0	-	N-EXP
		D483E	1	0	-	N-EXP
		E815D	1	0	-	N-EXP
RIFAMYCINS	*rpoB*	D355E	2	0	-	N-EXP
		p.D355E	1	0	-	N-EXP
TETRACYCLINES	*tet(38)*	-	7	1	-	ACC[8]
	mepA	-	1	-	-	OOS[9]
TRIMETHOPRIM	*dfrB*	I97T	2	0	-	N-EXP
		V72E	1	0	-	N-EXP
		V72E,I97T	1	0	-	N-EXP
		p.I97T	1	0	-	N-EXP
		p.V72E	1	0	-	N-EXP

3.9. Submitted AMR phenotype for *S. aureus* strain GPT24-03

Table 17. Submitted predicted AMR phenotype for *S. aureus* strain GPT24-03.					
Class (A-Z)	**Predicted Resistant Phenotype to Antimicrobial**	**No of Laboratories**	**Score if submitted**	**Score if not submitted**	**Scoring Category**
BETA-LACTAMS	Cefoxitin	31	1	0	EXP
	Penicillin	30	1	0	EXP
TETRACYCLINES	Tetracycline	7	1	-	ACC[8]

[8] The Tetracycline efflux MFS transporter Tet(38) is a chromosomally encoded, native efflux pump in *S. aureus*. Scientific literature suggests that only when the *tet(38)* gene is overexpressed it confers an increase in MIC for tetracycline (11–13). Moreover, this gene is reported by AMRFinderPlus but not by ResFinder. Therefore, reporting *tet(38)* gene is not penalized and both tetracycline-susceptible and tetracycline-resistant phenotypes are accepted.

[9] The gene *mepA*, encoding a MATE-family efflux pump associated with tigecycline resistance in *S. aureus* was detected with 100% coverage and 98.5% identity on the chromosome of *S. aureus* strain GPT24-03. However, since tigecycline is not included in the antimicrobial panel for *S. aureus* (**Table 2**) in this proficiency test, *mepA* is considered out of scope (OOS) for scoring purposes.

3.10. Expected AMR data for *S. aureus* strain GPT24-04

Table 18. Expected results for *S. aureus* strain GPT24-04. Predicted resistant phenotypes for specific antimicrobials are indicated in **bold**. Percent identity and coverage were calculated at protein level.

Class (A-Z)	Antimicrobial	Predicted AMR Phenotype	AMR Gene	AMR Mutation	Protein name	%Identity	%Coverage
AMINOGLYCOSIDES	Gentamicin	Susceptible	-	-	-	-	-
	Streptomycin						
	Kanamycin	**Resistant or** Susceptible[10]	Optional: *aadD1* [10]	-	**Aminoglycoside O-nucleotidyltransferase ANT(4')-Ia**	**100.0**	**100.0**
BETA-LACTAMS	**Penicillin**	**Resistant**	***blaZ***	-	**Penicillin-hydrolyzing class A beta-lactamase BlaZ**	**100.0**	**100.0**
	Cefoxitin	Susceptible	-	-	-	-	-
GLYCOPEPTIDES	Vancomycin	Susceptible	-	-	-	-	-
LINCOSAMIDES	**Clindamycin**	**Resistant**	***erm(C)***		**23S rRNA (adenine(2058)-N(6))-methyltransferase Erm(C)**	**99.2**[11]	**100.0**
MACROLIDES	**Erythromycin**	**Resistant**	***erm(C)***		**23S rRNA (adenine(2058)-N(6))-methyltransferase Erm(C)**	**99.2**[11]	**100.0**
OXAZOLIDINONES	Linezolid	Susceptible	-	-	-	-	-
PHENICOLS	Chloramphenicol	Susceptible	-	-	-	-	-
PLEUROMUTILINS	Tiamulin	Susceptible	-	-	-	-	-
PSEUDOMONIC ACIDS	Mupirocin	Susceptible[12]	Optional: *mupA*[12]	-	Mupirocin-resistant isoleucine--tRNA ligase MupA	100	91.1[12]

[10] Resistance to kanamycin conferred by the aminoglycoside O-nucleotidyltransferase ANT(4')-Ia, encoded by the *aadD1* gene, is reported by AMRFinderPlus but not by ResFinder. Therefore, omission of the *aadD1* gene or the corresponding predicted resistance to kanamycin is not penalised in the current proficiency test.

[11] Lower percent identity due to two amino acid substitutions (V19I and S101N) compared to the reference protein WP_014158490.1. The impact of these mutations on the functionality of the protein is unknown; however, for the purpose of this Genomic PT the gene is considered functional.

Table 18. Expected results for *S. aureus* strain GPT24-04. Predicted resistant phenotypes for specific antimicrobials are indicated in **bold**. Percent identity and coverage were calculated at protein level.

Class (A-Z)	Antimicrobial	Predicted AMR Phenotype	AMR Gene	AMR Mutation	Protein name	%Identity	%Coverage
QUINOLONES	**Ciprofloxacin**	**Resistant**	***grlA/parC***[13]	**S80F**	***Staphylococcus aureus* quinolone resistant ParC**	**99.3**[13]	**100.0**
RIFAMYCINS	Rifampin	Susceptible	-	-	-	-	-
STEROID ANTIBACTERIALS	**Fusidic acid**	**Resistant**	***fusB***	-	**Fusidic acid resistance EF-G-binding protein FusB**	**100.0**	**100.0**
STREPTOGRAMINS	Quinupristin/ dalfopristin	Susceptible	-	-	-	-	-
SULFONAMIDES	Sulfamethoxazole	Susceptible	-	-	-	-	-
TETRACYCLINES	**Tetracycline**	**Resistant**	***tet(L)***	-	**Tetracycline efflux MFS transporter Tet(L)**	**100.0**	**100.0**
TRIMETHOPRIM	Trimethoprim	Susceptible	-	-	-	-	-

[12] For the Mupirocin-resistant isoleucine--tRNA ligase MupA gene, *mupA*, a deletion of adenine at position 272 (272delA) causes a frameshift in the coding sequence, introducing a premature stop codon at position 320 - see alignment below. This results in a truncated protein, which is not expected to be functional. Consequently, the presence of *mupA* gene was not penalised, and the strain was considered susceptible to mupirocin. The observed 91.1% gene coverage was explained by an alternative downstream open reading frame, initiated at a start codon (ATG) located after the premature stop codon, at position 412. The alignment algorithm included this downstream region in the reported match, even though translation was unlikely to produce a functional protein beyond the truncated coding sequence caused by the frameshift.

[13] There are six amino acid substitutions compared to the reference protein WP_001289586.1 (S80F, S144P, I223V, Y410F, Y521F, I656V) therefore the lower percent identity.

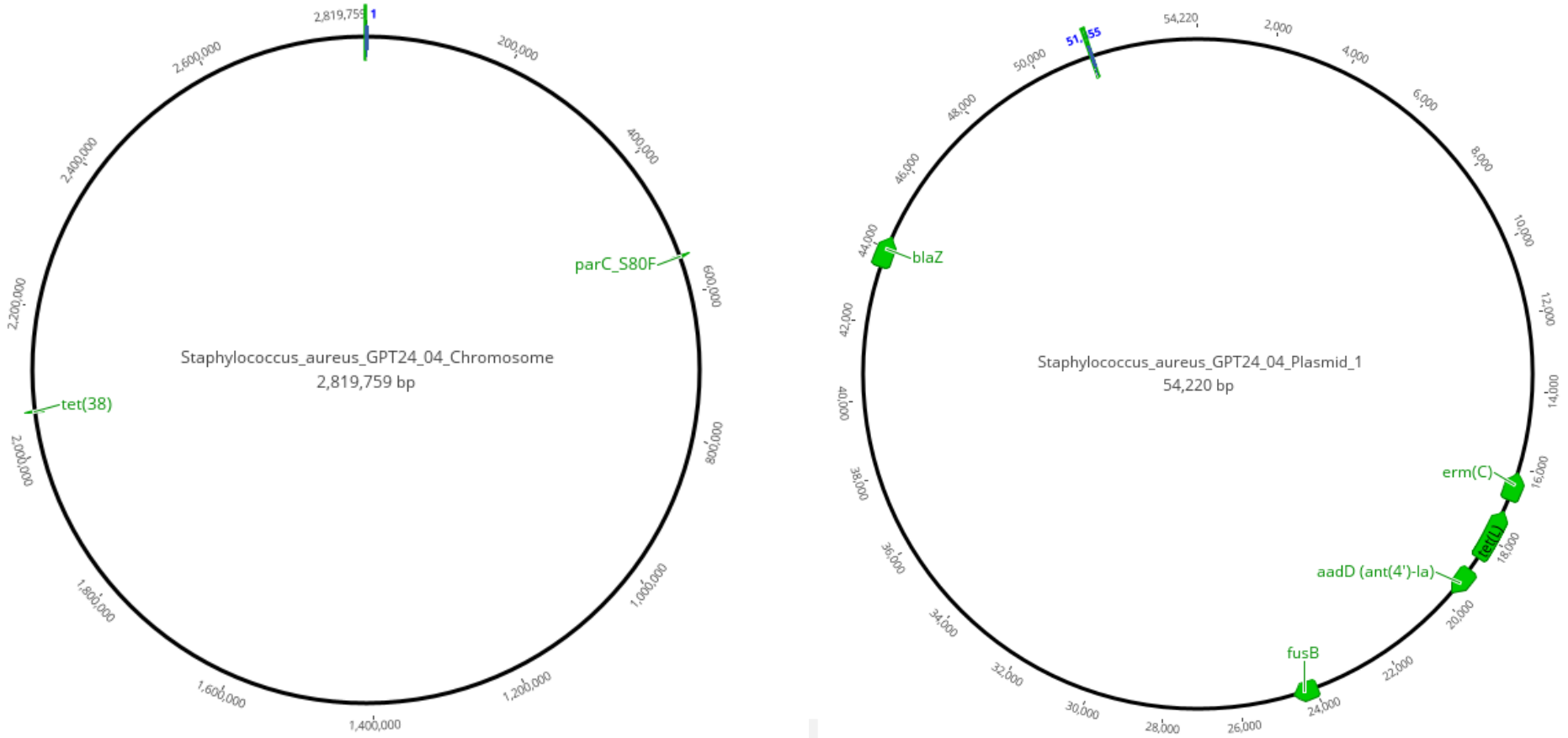

Figure 4. *Location of AMR genes or genes with mutations associated to AMR in S. aureus strain GPT24-04.* ***Left****: Chromosome,* ***Right****: Plasmid_1.*

3.11. Submitted AMR genes and mutations for *S. aureus* strain GPT24-04

Table 19. Submitted AMR genes for *S. aureus* strain GPT24-04.

Class (A-Z)	AMR Gene	AMR mutation	No of Laboratories	Score if submitted	Score if not submitted	Scoring Category
AMINOGLYCOSIDES	*aadD*	-	9	1	-	ACC[10]
	aadD1	-	5	1	-	ACC[10]
	aadA1	-	1	0	-	N-EXP
	aph(3')-III	-	1	0	-	N-EXP
	aph(3')-IIIa	-	1	0	-	N-EXP
BETA-LACTAMS	*blaZ*	-	31	1	0	EXP
	pbp4	p.T409A	1	0	-	N-EXP
		p.L234H	1	0	-	N-EXP
		T25A,T189S,L234H, T409A(Promoter: n.-54A>G,n.-60A>G,p.T25A)	1	0	-	N-EXP
		p.T189S	1	0	-	N-EXP
		p.T25A	1	0	-	N-EXP
	pbp2	p.E315A	1	0	-	N-EXP
		p.A606D	1	0	-	N-EXP
		E315A,A576S,A606D	1	0	-	N-EXP
		p.A576S	1	0	-	N-EXP
GLYCOPEPTIDES	*bleO*	-	5	-	-	OOS[14]
LINCOSAMIDES	*erm(C)*	-	19	1	0	EXP
	erm(B)	-	1	0	-	N-EXP
MACROLIDES	*erm(C)*	-	25	1	0	EXP
	erm(30)	-	1	0	-	N-EXP
PSEUDOMONIC ACIDS	*mupA*	-	25	1	-	ACC[12]
	ileS	N213D,K438E	1	0	-	N-EXP
		p.N213D	1	0	-	N-EXP
		p.K438E	1	0	-	N-EXP
QUINOLONES	*grlA/parC*	S80F	21	1	0	EXP
		p.S80F	1	1	-	FORM[4]
		grlA_p.S80F	1	1	-	FORM[4]
		grlAp.S80F	1	1	-	FORM[4]
		p.S80F,parC_S80F, tcc>ttc,s->f	1	1	-	FORM[4]
		grlA_S80F	1	1	-	FORM[4]
		p.S80F	4	1	-	FORM[4]
		p.V694M	1	0	-	N-EXP
		I223V,Y410F,V694M	1	0	-	N-EXP
		p.Y410F	1	0	-	N-EXP
		p.I223V	1	0	-	N-EXP
	grlB	H478Y,D530G	1	0	-	N-EXP
		p.H478Y	1	0	-	N-EXP
		p.D530G	1	0	-	N-EXP
	gyrA	A709E,D856E,N860T, E862D,S884L	1	0	-	N-EXP
		p.S80F	1	0	-	N-EXP

[14] The *bleO* gene encodes a bleomycin-binding protein that confers resistance to the glycopeptide antibiotic bleomycin. In the context of the DTU Genomic PT, bleomycin is not included in the antimicrobial panel for *S. aureus* (**Table 2**). Therefore, despite the detection of *bleO* with 100% identity and coverage on Plasmid 1, this gene is classified as out of scope (OOS) for this analysis.

Table 19. Submitted AMR genes for *S. aureus* strain GPT24-04.						
Class (A-Z)	**AMR Gene**	**AMR mutation**	**No of Laboratories**	**Score if submitted**	**Score if not submitted**	**Scoring Category**
		p.N860T	1	0	-	N-EXP
		p.A709E	1	0	-	N-EXP
		p.S884L	1	0	-	N-EXP
		p.D856E	1	0	-	N-EXP
		p.E862D	1	0	-	N-EXP
STEROID ANTIBACTERIALS	*fusB*	-	30	1	0	EXP
STREPTOGRAMINS	*erm(C)*		10	0	-	N-EXP[15]
TETRACYCLINES	*tet(L)*	-	29	1	0	EXP
	tet(38)	-	6	1	-	ACC[8]
	tet(O)	-	1	0	-	N-EXP
	tet(C)	-	1	0	-	N-EXP
TRIMETHOPRIM	*dfrB*	p.I97T	1	0	-	N-EXP
		V72E,I97T	1	0	-	N-EXP
		p.V72E	1	0	-	N-EXP

3.12. Submitted AMR phenotype for *S. aureus* strain GPT24-04

Table 20. Submitted predicted AMR phenotype for *S. aureus* strain GPT24-04.					
Class (A-Z)	**Predicted Resistant Phenotype to Antimicrobial**	**No of Laboratories**	**Score if submitted**	**Score if not submitted**	**Scoring Category**
AMINOGLYCOSIDES	Kanamycin	4	1	-	ACC[10]
BETA-LACTAMS	Cefoxitin	1	0	-	N-EXP
	Penicillin	29	1	0	EXP
LINCOSAMIDES	Clindamycin	28	1	0	EXP
MACROLIDES	Erythromycin	29	1	0	EXP
PSEUDOMONIC ACIDS	Mupirocin	23	0	-	N-EXP[12]
QUINOLONES	Ciprofloxacin	24	1	0	EXP
STEROID ANTIBACTERIALS	Fusidic acid	27	1	0	EXP
STREPTOGRAMINS	Quinupristin-dalfopristin	9	0	-	N-EXP[15]
TETRACYCLINES	Tetracycline	30	1	0	EXP

[15] *S. aureus* GPT24-04 is predicted to be susceptible to quinupristin-dalfopristin (Q-D). This strain harbors the *ermC* gene which encodes a methyltransferase that methylates the 23S rRNA, leading to resistance against macrolides, lincosamides, and streptogramin B antibiotics. However, Q-D is a synergistic combination of streptogramin B (quinupristin) and streptogramin A (dalfopristin). Resistance to Q-D typically requires mechanisms that affect both components. In the absence of additional resistance determinants targeting streptogramin A, the presence of *erm(C)* alone is not predicted to confer high-level resistance to the Q-D combination.

3.13. Expected AMR data for *E. faecalis* strain GPT24-05

Table 21 . Expected results for *E. faecalis* strain GPT24-05. Predicted resistant phenotypes for specific antimicrobials are indicated in **bold**. Percent identity and coverage were calculated at protein level.

Class (A-Z)	Antimicrobial	Predicted AMR Phenotype	AMR Gene	AMR Mutation	Protein name	%Identity	%Coverage
AMINOGLYCOSIDES	Gentamicin	**Expected Resistant Phenotype**[16]	-[17]	-	-	-	-
BETA-LACTAMS	Ampicillin	Susceptible	-	-	-	-	-
GLYCOPEPTIDES	Teicoplanin	Susceptible	-	-	-	-	-
	Vancomycin						
LIPOPEPTIDES	Daptomycin	Susceptible	-	-	-	-	-
MACROLIDES	**Erythromycin**	**Expected Resistant Phenotype**[16]	Optional: ***erm(B)***[18]	-	**23S rRNA (adenine(2058)-N(6))-methyltransferase Erm(B)**	**99.6**[19]	**100.0**
OXAZOLIDINONES	**Linezolid**	**Resistant**	***optrA***	-	**ABC-F type ribosomal protection protein OptrA**	**99.9**[19]	**100.0**
PHENICOLS	**Chloramphenicol**	**Resistant**	***fexA***	-	**Chloramphenicol/florfenicol efflux MFS transporter FexA**	**99.0**[19]	**100.0**
			optrA	-	**ABC-F type ribosomal protection protein OptrA**	**99.9**[19]	**100.0**
QUINOLONES	Ciprofloxacin	Susceptible	-	-	-	-	-
STREPTOGRAMINS	**Quinupristin-dalfopristin**	**Expected Resistant Phenotype**[16]	Optional: ***lsa(A)***[20]	-	**ABC-F type ribosomal protection protein Lsa(A)**	**99.2**[20]	**100.0**
TETRACYCLINES	**Tetracycline**	**Resistant**	***tet(L)***	-	**Tetracycline efflux MFS transporter Tet(L)**	**100.0**	**100.0**
			tet(M)	-	**Tetracycline resistance ribosomal protection protein Tet(M)**	**100.0**	**100.0**
	Tigecycline	Susceptible	-	-	-	-	-

[16] We acknowledge that the EUCAST "Expected Resistant Phenotypes" document (Version 1.2, January 2023; available at EUCAST) lists aminoglycosides (low-level resistance expected), macrolides, and quinupristin–dalfopristin (resistance expected) for *E. faecalis*. Laboratories are expected to either **not report a result** at all, or to **report the isolate as resistant**. For the DTU Genomic PT 2024, results for aminoglycosides (gentamicin), macrolides (erythromycin), and quinupristin–dalfopristin are **excluded from the analysis** – see **Scope** and **Scoring Categories**.

[17] Enterococci are thought to exhibit low-level resistance to aminoglycosides due to limiting drug uptake, therefore the absence of a specific genetic determinant (14).

[18] The strain carries the *erm(B)* gene, encoding a 23S rRNA methyltransferase that confers high-level macrolide resistance. Although *erm(B)* is an acquired gene, often integrated into the chromosome via mobile genetic elements, it is highly prevalent among *E. faecalis* isolates; therefore, EUCAST classifies erythromycin resistance as an expected resistant phenotype.

[19] The observed lower percent identities for *erm(B)*, *optrA*, and *fexA* are each attributed to specific amino acid substitutions: V226I in *erm(B)*, Y176D in *optrA*, and five substitutions in *fexA* (A34S, L39S, S50A, I131V, V305I). The impact of these substitutions to the protein functionality is unknown. However, in the context of the DTU Genomic PT, all three proteins are considered functional. Accordingly, the strain is classified as resistant to erythromycin, linezolid and chloramphenicol.

[20] *E. faecalis* has an expected resistant phenotype to quinupristin-dalfopristin via the *lsa* gene, coding for an ABC type ribosomal protection protein (15,16). The percent identity is lower due to 10 nucleotide substitutions in the genome of strain GPT24-05 compared to the reference *lsa* gene with accession number AY225127, leading to four amino acid substitutions in the corresponding protein. The effect of these amino acid substitutions to the protein functionality is unknown. For the purpose of the DTU Genomic PT 2024, the protein is considered functional in this strain.

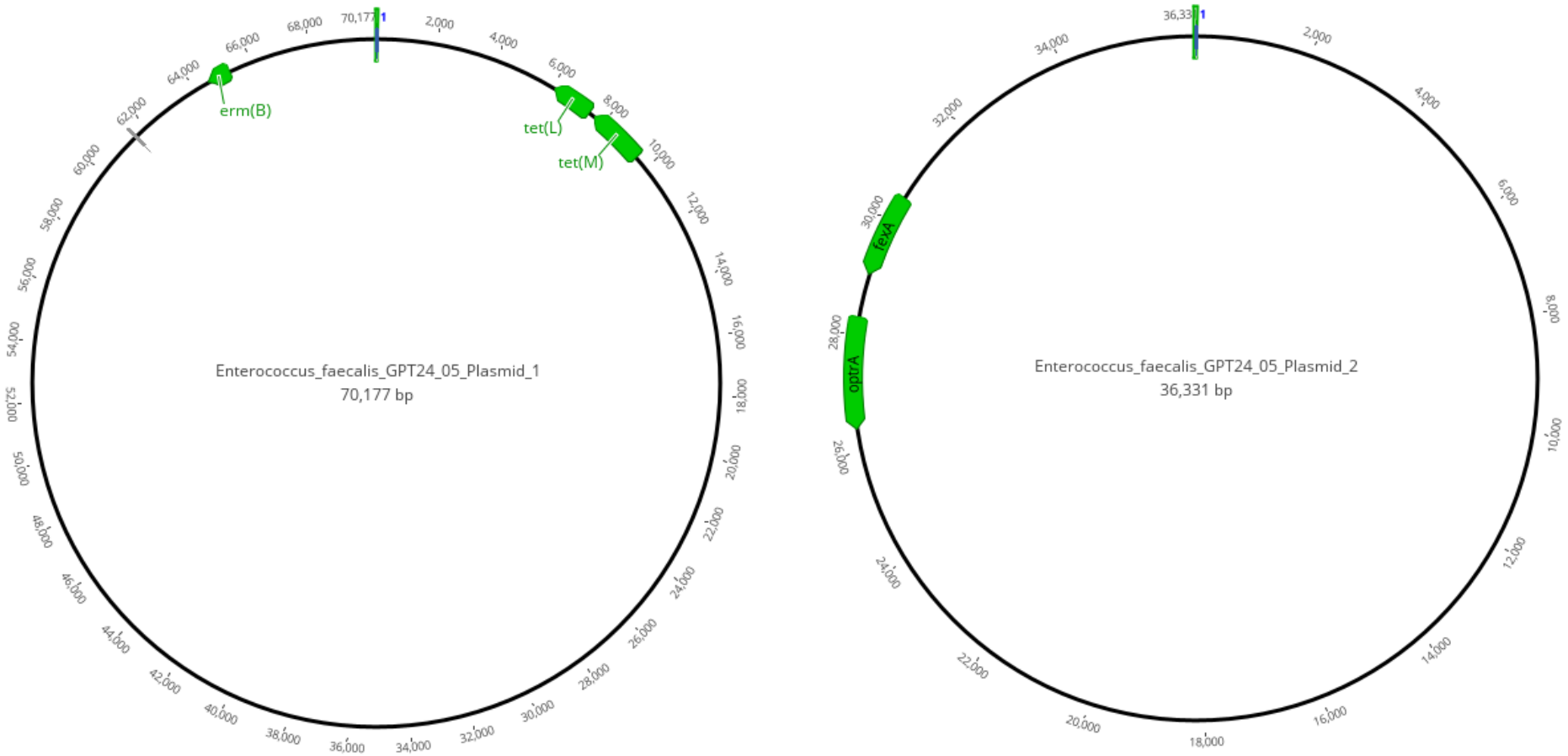

***Figure 5**. Location of AMR genes or genes with mutations associated to AMR in E. faecalis strain GPT24-05. **Left**: Plasmid_1, **Right**: Plasmid_2. The ABC-F type ribosomal protection protein Lsa(A) gene lsa(A) is located on the chromosome of the strain at position 2255118 (not shown).*

3.14. Submitted AMR genes and mutations for *E. faecalis* GPT24-05

Table 22. Submitted AMR genes for *E. faecalis* strain GPT24-05.						
Class (A-Z)	**AMR Gene**	**AMR mutation**	**No of Laboratories**	**Score if submitted**	**Score if not submitted**	**Scoring Category**
AMINOGLYCOSIDES	*str*	-	6	-	-	NAR[21]
MACROLIDES	*erm(B)*	-	30	-	-	NAR[16,18]
OXAZOLIDINONES	*optrA*	-	23	1	0	EXP
PHENICOLS	*fexA*	-	25	1	0	EXP
	optrA	-	18	1	0	EXP
QUINOLONES	*parC*	p.V143I	3	0	-	N-EXP
STREPTOGRAMINS	*erm(B)*		9	0	-	N-EXP[22]
TETRACYCLINES	*tet(45)*	-	1	0	-	N-EXP
	tet(L)	-	26	1	0	EXP
	tet(M)	-	23	1	0	EXP

3.15. Submitted AMR phenotype for *E. faecalis* strain GPT24-05

Table 23. Submitted predicted AMR phenotype for *E. faecalis* strain GPT24-05.					
Class (A-Z)	**Predicted Resistant Phenotype to Antimicrobial**	**No of Laboratories**	**Score if submitted**	**Score if not submitted**	**Scoring Category**
MACROLIDES	Erythromycin	29	-	-	NAR[16,18]
PHENICOLS	Chloramphenicol	28	1	0	EXP
OXAZOLIDINONES	Linezolid	27	1	0	EXP
STREPTOGRAMINS	Quinupristin-dalfopristin	14	-	-	NAR[20,22]
TETRACYCLINES	Tetracycline	28	1	0	EXP
	Tigecycline	6	0	-	N-EXP

[21] The *str* gene is located on Plasmid_1 with 100% identity and coverage and is predicted to confer resistance to the aminoglycoside streptomycin (not included in the panel for *E. faecalis* in the DTU Genomic PT - **Table 2**). *E. faecalis* is expected to have low level resistance to aminoglycosides – see footnote 16.

[22] This strain carries the *erm(B)* gene, which encodes an rRNA methyltransferase that methylates the 23S rRNA, conferring resistance to macrolides, lincosamides, and streptogramin B antibiotics. Quinupristin–dalfopristin (Q–D) is a synergistic combination of streptogramin A and B compounds and is the only representative of the streptogramin class included in the *E. faecalis* antimicrobial panel (**Table 2**). The presence of *erm(B)* does not contribute to resistance to Q–D. As in most *E. faecalis* isolates, this strain also harbors the *lsa(A)* gene, encoding an ABC-F type ribosomal protection protein (LsaA) responsible for resistance to Q–D. *E. faecalis* is therefore expected to be resistant to quinupristin–dalfopristin according to EUCAST guidelines for expected resistant phenotypes (Version 1.2, January 2023; available at EUCAST).

3.16. Expected AMR data for *E. faecium* strain GPT24-06

Table 24. Expected results for *E. faecium* strain GPT24-06. Predicted resistant phenotypes for specific antimicrobials are indicated in **bold**.
Percent identity and coverage were calculated at protein level.

Class (A-Z)	Antimicrobial	Predicted AMR Phenotype	AMR Gene	AMR Mutation	Protein name	%Identity	%Coverage
AMINOGLYCOSIDES	Gentamicin	**Expected Resistant Phenotype**[23]	-[24]	-	-	-	-
BETA-LACTAMS	**Ampicillin**	**Resistant**[25]	***pbp5***	**M485A E629V V586L**	***Enterococcus faecium* beta-lactam resistant Pbp5**	**97.1**	**100.0**
GLYCOPEPTIDES	Teicoplanin	Susceptible	None detected	-	-	-	-
	Vancomycin	**Resistant**	***vanH-B*** ***vanB*** ***vanX-B***[26]	**-**	**D-lactate dehydrogenase VanH-B** **D-alanine--(R)-lactate ligase VanB** **D-Ala-D-Ala dipeptidase VanX-B**	**100.0**	**100.0**
LIPOPEPTIDES	Daptomycin	Susceptible	-	-	-	-	-
MACROLIDES	**Erythromycin**	**Expected Resistant Phenotype**[23]	Optional: ***msr(C)***[27]	**-**	**ABC-F type ribosomal protection protein Msr(C)**	**99.0**	**100.0**

[23] We acknowledge that the EUCAST "Expected Resistant Phenotypes" document (Version 1.2, January 2023; available at EUCAST) lists aminoglycosides (low-level resistance expected), and macrolides (resistance expected) for *E. faecium*. Laboratories are expected to either **not report a result** at all, or to **report the isolate as resistant**. For the DTU Genomic PT 2024, results for aminoglycosides (gentamicin) and macrolides (erythromycin) are **excluded from the analysis** – see **Scope** and **Scoring Categories**.

[24] Enterococci are thought to exhibit low-level resistance to aminoglycosides due to limiting drug uptake, therefore the absence of a specific genetic determinant (14).

[25] Ampicillin resistance in *E. faecium* is associated with point mutations in the penicillin-binding protein gene pbp5, which result in amino acid substitutions that reduce the binding affinity of PBP5 for ampicillin. Two allelic variants of pbp5 have been described, differing by approximately 5% at the nucleotide level and encoding two distinct protein isoforms: PBP5-S (susceptible) and PBP5-R (resistant). The PBP5-R variant includes 20 defined amino acid substitutions: V24A, S27G, R34Q, G66E, A68T, E85D, E100Q, K144Q, T172A, L177I, D204G, A216S, T324A, M485A, N496K, A499T, E525D, V586L, E629V, and P667S (17). ResFinder reports all 20 substitutions, while AMRFinderPlus reports a subset (M485A, V586L, and E629V). These substitutions are known to act synergistically in conferring resistance; therefore, reporting any of these mutations is accepted.

[26] The core resistance enzymes of the *vanB*-type glycopeptide resistance operon include *vanH-B*, *vanB*, and *vanX-B*. AMRFinderPlus reports these genes individually, whereas ResFinder denotes the operon collectively as *vanHBX*. Both formats are acceptable for reporting, as they represent the same resistance mechanisms.

[27] ABC-F type ribosomal protection protein Msr(C) is considered intrinsic, *i.e.*, it is present in most of *E. faecium* isolates. It is predicted to confer natural resistance to macrolides, including erythromycin as well as to streptogramin B antibiotics. The lower percent identity observed for the *msr(C)* gene is due to five amino acid substitutions: D365G, V374I, A404T, M419T, and V454I. The impact of these substitutions on protein function is unknown; however, in the context of the DTU Genomic PT the gene is considered to code for a functional protein.

Table 24. Expected results for *E. faecium* strain GPT24-06. Predicted resistant phenotypes for specific antimicrobials are indicated in **bold**.
Percent identity and coverage were calculated at protein level.

Class (A-Z)	Antimicrobial	Predicted AMR Phenotype	AMR Gene	AMR Mutation	Protein name	%Identity	%Coverage
OXAZOLIDINONES	Linezolid	Susceptible	None detected	-	-	-	-
PHENICOLS	Chloramphenicol	Susceptible	None detected	-	-	-	-
QUINOLONES	**Ciprofloxacin**	**Resistant**	***gyrA***	**S83Y**	***Enterococcus faecium* quinolone resistant GyrA**	**99.6**	**100.0**
			parC	**S80I**	***Enterococcus faecium* quinolone resistant ParC**	**99.8**	**100.0**
STREPTOGRAMINS	Quinupristin/ Dalfopristin	Susceptible	None detected	-	-	-	-
TETRACYCLINES	**Tetracycline**	**Resistant**	***tet(M)***	**-**	**Tetracycline resistance ribosomal protection protein Tet(M)**	**100.0**	**100.0**
	Tigecycline	Susceptible	None detected	-	-	-	-

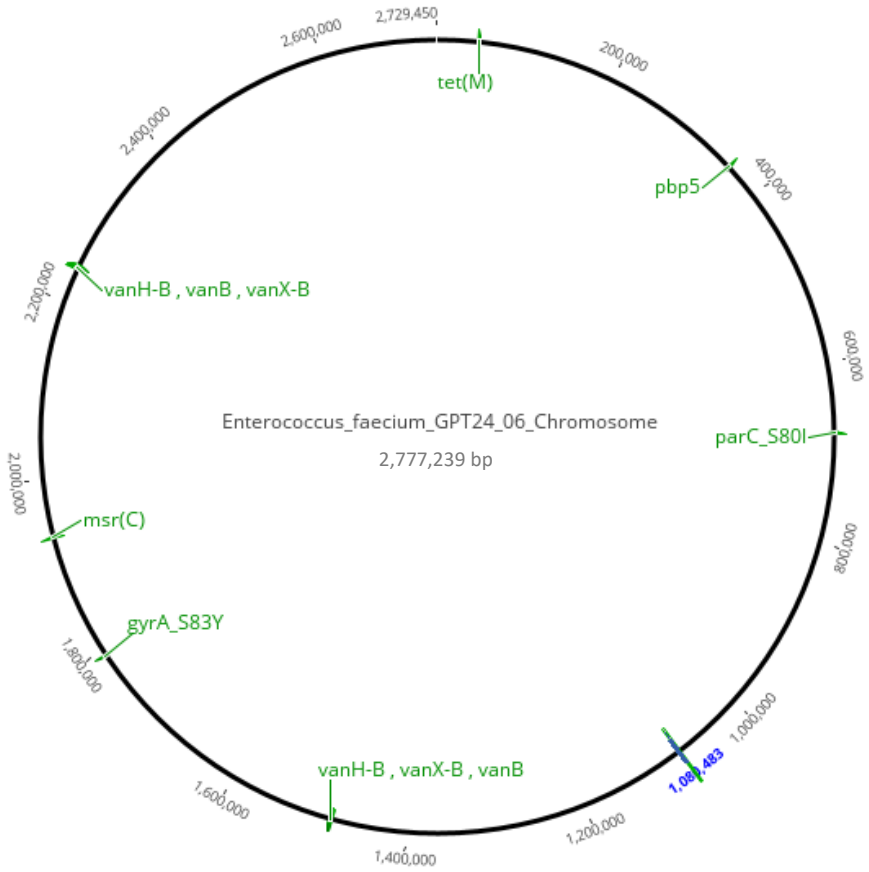

***Figure 6**. Location of AMR genes or genes with mutations associated to AMR in E. faecium strain GPT24-06 chromosome.*

3.17. Submitted AMR genes and mutations for *E. faecium* GPT24-06

Table 25. Submitted AMR genes for *E. faecium* stain GPT24-06.						
Class (A-Z)	**AMR Gene**	**AMR mutation**	**No of Laboratories**	**Score if submitted**	**Score if not submitted**	**Scoring Category**
AMINOGLYCOSIDES	*aac(6')-Ii*	-	22	-	-	NAR[28]
	aac(6')-I	-	5			
BETA-LACTAMS	pbp5	M485A	17	1	0	EXP
		E629V	14	1	0	EXP
		V586L	14	1	0	EXP
		p.M485A	2	1	-	FORM[4]
		p.E629V	2	1	-	FORM[4]
		p.V586L	2	1	-	FORM[4]
		p.M485A	1	1	-	FORM[4]
		pbp5_M485A	1	1	-	FORM[4]
		p.M485A,atg>gcg,m>a, ampicillin, PMID 25182648	1	1	-	FORM[4]
		pbp5p.M485A	1	1	-	FORM[4]
		pbp5_p.M485A	1	1	-	FORM[4]
		p.E629V	1	1	-	FORM[4]
		p.E629V,gaa>gta,e>v, ampicillin, PMID 25182648	1	1	-	FORM[4]
		pbp5_p.E629V	1	1	-	FORM[4]
		pbp5p.E629V	1	1	-	FORM[4]
		p.V586L	1	1	-	FORM[4]
		pbp5p.V586L	1	1	-	FORM[4]
		p.V586L,gta>tta,v>l, ampicillin, PMID 25182648	1	1	-	FORM[4]
		pbp5_p.V586L	1	1	-	FORM[4]
		E629V;M485A;V586L	1	1	-	FORM[4]
		T324A	13	1	-	ACC[25]
		P667S	13	1	-	ACC[25]
		N496K	13	1	-	ACC[25]
		E100Q	13	1	-	ACC[25]
		S27G	13	1	-	ACC[25]
		E525D	13	1	-	ACC[25]
		A68T	13	1	-	ACC[25]
		A499T	13	1	-	ACC[25]
		D204G	13	1	-	ACC[25]
		E85D	13	1	-	ACC[25]
		R34Q	13	1	-	ACC[25]
		G66E	13	1	-	ACC[25]
		T172A	13	1	-	ACC[25]
		K144Q	13	1	-	ACC[25]
		L177I	13	1	-	ACC[25]
		A216S	13	1	-	ACC[25]

[28] Some species of *Enterococcus*, among them *E. faecium*, harbor on the chromosome the intrinsic gene *aac(6')-I* (or *aac(6')-Ii*) coding for an aminoglycoside 6'-N-acetyltransferase, conferring resistance to tobramycin, kanamycin and amikacin. This gene is present in *E. faecium* strain GPT24-06 with 100% identity and coverage.

Table 25. Submitted AMR genes for *E. faecium* stain GPT24-06.						
Class (A-Z)	**AMR Gene**	**AMR mutation**	**No of Laboratories**	**Score if submitted**	**Score if not submitted**	**Scoring Category**
		V24A	12	1	-	ACC[25]
		p.V24A	3	1	-	ACC[25]
		p.D204G	2	1	-	ACC[25]
		p.E100Q	2	1	-	ACC[25]
		p.A68T	2	1	-	ACC[25]
		p.E525D	2	1	-	ACC[25]
		p.A216S	2	1	-	ACC[25]
		p.E85D	2	1	-	ACC[25]
		p.G66E	2	1	-	ACC[25]
		p.K144Q	2	1	-	ACC[25]
		p.L177I	2	1	-	ACC[25]
		p.N496K	2	1	-	ACC[25]
		p.P667S	2	1	-	ACC[25]
		p.R34Q	2	1	-	ACC[25]
		p.S27G	2	1	-	ACC[25]
		p.T324A	2	1	-	ACC[25]
		p.A499T	2	1	-	ACC[25]
		p.T172A	2	1	-	ACC[25]
		pbp5_p.A216S	1	1	-	ACC[25]
		pbp5p.P667S	1	1	-	ACC[25]
		pbp5_p.T172A	1	1	-	ACC[25]
		p.A68T	1	1	-	ACC[25]
		p.V24A,p.S27G,p.R34Q, p.G66E, p.A68T, p.E85D, p.E100Q, p.K144Q, P.T172A,	1	1	-	ACC[25]
		p.A68T,gca>aca,a>t, ampicillin, PMID 25182648	1	1	-	ACC[25]
		pbp5_p.G66E	1	1	-	ACC[25]
		pbp5p.E100Q	1	1	-	ACC[25]
		p.D204G	1	1	-	ACC[25]
		p.A216S,gca>tcc,a>s, ampicillin, PMID 25182648	1	1	-	ACC[25]
		p.D204G,gac>ggc,d>g, ampicillin, PMID 25182648	1	1	-	ACC[25]
		p.D644N	1	1	-	ACC[25]
		pbp5_p.E100Q	1	1	-	ACC[25]
		pbp5_p.N496K	1	1	-	ACC[25]
		p.E100Q	1	1	-	ACC[25]
		pbp5p.A216S	1	1	-	ACC[25]
		p.E100Q,gag>cag,e>q, ampicillin, PMID 25182648	1	1	-	ACC[25]
		pbp5p.T324A	1	1	-	ACC[25]
		p.E525D	1	1	-	ACC[25]
		p.A499T	1	1	-	ACC[25]
		p.E525D,gag>gat,e>d, ampicillin, PMID 25182648	1	1	-	ACC[25]

Table 25. Submitted AMR genes for *E. faecium* stain GPT24-06.						
Class (A-Z)	**AMR Gene**	**AMR mutation**	**No of Laboratories**	**Score if submitted**	**Score if not submitted**	**Scoring Category**
		p.V24A,p.S27G;p.R34Q; p.G66E;p.A68T;p.E85D; p.E100Q;p.K144Q;p.T172A;	1	1	-	ACC[25]
		pbp5_p.A68T	1	1	-	ACC[25]
		pbp5_p.L177I	1	1	-	ACC[25]
		p.E85D	1	1	-	ACC[25]
		pbp5_p.R34Q	1	1	-	ACC[25]
		p.E85D,gaa>gat,e>d, ampicillin, PMID 25182648	1	1	-	ACC[25]
		pbp5_p.V24A	1	1	-	ACC[25]
		pbp5p.A68T	1	1	-	ACC[25]
		p.G66E	1	1	-	ACC[25]
		p.G66E,gga>gaa,g>e, ampicillin, PMID25182648	1	1	-	ACC[25]
		pbp5p.S27G	1	1	-	ACC[25]
		p.K144Q	1	1	-	ACC[25]
		p.K144Q,aaa>caa,k>q, ampicillin, PMID 25182648	1	1	-	ACC[25]
		S83Y	1	0	-	N-EXP
		p.V24A,gta>gca,v>a, ampicillin, PMID 25182648	1	1	-	ACC[25]
		p.L177I	1	1	-	ACC[25]
		p.V24A,p.T25A,p.S27G	1	1	-	ACC[25]
		p.L177I,tta>ata,l>i, ampicillin, PMID 25182648	1	1	-	ACC[25]
		pbp5_p.A499T	1	1	-	ACC[25]
		pbp5_p.D204G	1	1	-	ACC[25]
		p.N496K	1	1	-	ACC[25]
		pbp5_p.E525D	1	1	-	ACC[25]
		p.N496K,aat>aaa,n>k, ampicillin, PMID 25182648	1	1	-	ACC[25]
		pbp5_p.E85D	1	1	-	ACC[25]
		pbp5_p.K144Q	1	1	-	ACC[25]
		p.P667S	1	1	-	ACC[25]
		p.P667S,ccc>tcg,p>s, ampicillin, PMID 25182648	1	1	-	ACC[25]
		pbp5_p.P667S	1	1	-	ACC[25]
		p.Q461K	1	0	-	N-EXP
		pbp5_p.S27G	1	1	-	ACC[25]
		pbp5_p.T324A	1	1	-	ACC[25]
		p.R34Q	1	1	-	ACC[25]
		p.R34Q,cgg>cag,r>q, ampicillin, PMID 25182648	1	1	-	ACC[25]
		pbp5p.A499T	1	1	-	ACC[25]

Table 25. Submitted AMR genes for *E. faecium* stain GPT24-06.						
Class (A-Z)	**AMR Gene**	**AMR mutation**	**No of Laboratories**	**Score if submitted**	**Score if not submitted**	**Scoring Category**
		pbp5p.D204G	1	1	-	ACC[25]
		p.S27G	1	1	-	ACC[25]
		pbp5p.E525D	1	1	-	ACC[25]
		p.S27G,agt>ggt,s->g, ampicillin, PMID 25182648	1	1	-	ACC[25]
		pbp5p.E85D	1	1	-	ACC[25]
		pbp5p.G66E	1	1	-	ACC[25]
		pbp5p.K144Q	1	1	-	ACC[25]
		pbp5p.L177I	1	1	-	ACC[25]
		pbp5p.N496K	1	1	-	ACC[25]
		p.T172A	1	1	-	ACC[25]
		pbp5p.R34Q	1	1	-	ACC[25]
		p.T172A,aca>gca,t->a, ampicillin, PMID 25182648	1	1	-	ACC[25]
		pbp5p.T172A	1	1	-	ACC[25]
		p.T25A	1	0	-	N-EXP
		pbp5p.V24A	1	1	-	ACC[25]
		p.A216S	1	1	-	ACC[25]
		p.T324A	1	1	-	ACC[25]
		S80I	1	0	-	N-EXP
		p.T324A,aca>gca,t->a, ampicillin, PMID 25182648	1	1	-	ACC[25]
		p.A499T,gca>aca,a->t, ampicillin, PMID 25182648	1	1	-	ACC[25]
		p.V24A	1	1	-	ACC[25]
		p.S39T	1	0	-	N-EXP
GLYCOPEPTIDES	*vanHBX*	-	26	1	0	EXP
	vanB	-	4	1	0	EXP
	vanX	-	3	1	0	EXP
	vanH	-	1	1	0	EXP
MACROLIDES	*msr(C)*	-	26	-	-	NAR[23,27]
QUINOLONES	*gyrA*[4]	S83Y	18	1	0	EXP
		p.S83Y	5	1	-	FORM[4]
		*gyrA*_p.S83Y	1	1	-	FORM[4]
		p.S83Y	1	1	-	FORM[4]
		gyrAp.S83Y	1	1	-	FORM[4]
		gyrA_S83Y	1	1	-	FORM[4]
		p.S83Y,agt>tat,s -> y, ciprofloxacin,nalidixic acid, PMID 12373497,9527801	1	1	-	FORM[4]
		p.83Y,p.N708D	1	0	-	N-EXP
		p.N708D	1	0	-	N-EXP
	parC	S80I	19	1	0	EXP
		p.S80I	6	1	-	FORM[4]
		parCp.S80I	1	1	-	FORM[4]

Table 25. Submitted AMR genes for *E. faecium* stain GPT24-06.						
Class (A-Z)	**AMR Gene**	**AMR mutation**	**No of Laboratories**	**Score if submitted**	**Score if not submitted**	**Scoring Category**
		p.S80I	1	1	-	FORM[4]
		S83Y	1	0	-	N-EXP
		parC_p.S80I	1	1	-	FORM[4]
		parC_S80I	1	1	-	FORM[4]
STREPTOGRAMINS	*msr(C)*	-	9	0	-	N-EXP[29]
TETRACYCLINES	*tet(M)*	-	30	1	0	EXP

3.18. Submitted AMR phenotype for *E. faecium* strain GPT24-06

Table 26. Submitted predicted AMR phenotype for *E. faecium* stain GPT24-06.					
Class (A-Z)	**Predicted Resistant Phenotype to Antimicrobial**	**No of Laboratories**	**Score if submitted**	**Score if not submitted**	**Scoring Category**
AMINOGLYCOSIDES	Gentamicin	23	-	-	NAR[23, 24]
BETA-LACTAMS	Ampicillin	22	1	0	EXP
GLYCOPEPTIDES	Vancomycin	26	1	0	EXP
MACROLIDES	Erythromycin	25	-	-	NAR[23, 27]
QUINOLONES	Ciprofloxacin	23	1	0	EXP
STREPTOGRAMINS	Quinupristin-dalfopristin	10	0	-	N-EXP[29]
TETRACYCLINES	Tetracycline	27	1	0	EXP

[29] This strain is predicted to be susceptible to quinupristin-dalfopristin (Q-D). It carries the intrinsic *msr(C)* gene, which encodes an ABC-F type ribosomal protection protein associated with resistance to macrolides and streptogramin B antibiotics. However, Q-D is a synergistic combination of streptogramin B (quinupristin) and streptogramin A (dalfopristin), and resistance typically requires mechanisms affecting both components. In the absence of additional resistance determinants targeting streptogramin A, the presence of *msr(C)* alone is not predicted to be sufficient to confer high-level resistance to the Q-D combination.

4. PLASMID REPLICON DATA

4.1. Expected Plasmid Replicon Data

The expected replicon types for each strain included in the DTU Genomic PT 2024 are presented in **Table 27**. Discrepancies between results obtained using PlasmidFinder and MOB-typer (part of the MOB-suite) may arise due to differences in their underlying databases and detection algorithms, which can affect both sensitivity and specificity. In addition, variations in identity and coverage thresholds—whether user-defined or tool-default—along with the choice of assembly and polishing tools, may further influence replicon detection. Unlike antimicrobial resistance, where genotypic data often directly correlates with a phenotypic outcome, such direct associations are not applied for plasmid replicons. As a result, one tool may detect replicons that another does not, or may classify them differently, leading to variations in the final analysis.

Table 27. Expected replicon types for each strain.

Organism – Strain	Location	Size (bp)	Known Replicon
E. coli – GPT24-01	Plasmid_1	132,041	IncFIB
	Plasmid_2	96,237	-
	Plasmid_3	32,937	IncX
	Plasmid_4	2,101	Col(BS512)
	Plasmid_5	1,549	-
E. coli – GPT24-02	Plasmid_1	130,631	IncFIB
	Plasmid_2	102,589	-
	Plasmid_3	46,125	-
	Plasmid_4	33,303	IncX
	Plasmid_5	7,176	-
	Plasmid_6	4,779	-
	Plasmid_7	4,315	-
	Plasmid_8	1,541	-
S. aureus – GPT24-03	Plasmid_1	3,710	-
S. aureus – GPT24-04	Plasmid_1	54,220	Rep3, rep5a RepA_N, rep20 Rep1, repUS12
E. faecalis - GPT24-05	Plasmid_1	70,177	Rep_trans, rep7a Rep_trans, repUS43 RepA_N, rep9b
	Plasmid_2	36,331	Rep3, repUS40
E. faecium - GPT24-06	Plasmid_1	208,043	RepA_N, repUS15
	Plasmid_3	10,366	Rep3, rep29

4.2. Submitted plasmid replicons for *E. coli* strain GPT24-01

Table 28. Submitted plasmid replicons for *E. coli* strain GPT24-01.

Replicon	No of laboratories	Score if reported	Score if not reported	Scoring Category
p0111	35	1	-	ACC[30]
Col(BS512)	33	1	0	EXP
FIB	32	1	0	EXP
X	27	1	0	EXP
FII	21	1	-	ACC[30]
Col(MG828)	12	1	-	ACC[30]
FIA	2	1	-	ACC[30]
HI1B	2	1	-	ACC[30]
FIC	2	1	-	ACC[30]

4.3. Submitted plasmid replicons for *E. coli* strain GPT24-02

Table 29. Submitted plasmid replicons for *E. coli* strain GPT24-02.

Replicon	No of laboratories	Score if reported	Score if not reported	Scoring Category
FII	32	1	-	ACC[31]
Col440II	30	1	-	ACC[31]
FIB	30	1	0	EXP
I1	29	1	-	ACC[31]
X	28	1	0	EXP
Col(MG828)	16	1	-	ACC[31]
Col(pHAD28)	9	1	-	ACC[31]
ColRNAI	6	1	-	ACC[31]
Iγ	2	1	-	ACC[31]
FIA	2	1	-	ACC[31]
FIC	2	1	-	ACC[31]
Col440I	4	0	-	N-EXP

[30] IncFII and p0111 were detected only by PlasmidFinder, while IncFIA, IncFIC and IncHI1B were identified only by MOB-suite. All replicon types are accepted. The Col(MG828) was identified by both PlasmidFinder and MOB-suite and was accepted although it only had 91.6% identity (below our defined threshold of 95%).

[31] IncFII, Col440II and IncI1 were detected only by PlasmidFinder, whereas IncFIA, IncFIC and IncIγ were identified only by MOB-suite. All replicon types are accepted. Col(MG828) was identified by both PlasmidFinder and MOB-suite and was accepted although it only had 94.2% identity (below our defined threshold of 95%). Col(pHAD28) and ColRNAI were detected only by PlasmidFinder, with a 90.8% identity, lower than our established threshold, however they are both accepted.

4.4. Submitted plasmid replicons for *S. aureus* strain GPT24-04

Table 30. Submitted plasmid replicons for *S. aureus strain* GPT24-04.

Replicon	No of laboratories	Score if reported	Score if not reported	Scoring Category
Rep1, repUS12	25	1	0	EXP
RepA_N, rep20	25	1	0	EXP
Rep3, rep5a	25	1	0	EXP
Rep3, rep6	2	0	-	N-EXP
Rep1, rep22	2	0	-	N-EXP

4.5. Submitted plasmid replicons for *E. faecalis* strain GPT24-05

Table 31. Submitted plasmid replicons for *E. faecalis* strain GPT24-05.

Replicon	No of laboratories	Score if reported	Score if not reported	Scoring Category
Rep_trans, repUS43	26	1	0	EXP
Rep3, repUS40	25	1	0	EXP
RepA_N, rep9b	20	1	0	EXP
Rep_trans, rep7a	16	1	0	EXP
Rep3, rep18a	1	0	-	N-EXP
RepA_N, rep9a	1	0	-	N-EXP
Rep3, rep18b	1	0	-	N-EXP

4.6. Submitted plasmid replicons for *E. faecium* strain GPT24-06

Table 32. Submitted plasmid replicons for *E. faecium* strain GPT24-06.

Replicon	No of laboratories	Score if reported	Score if not reported	Scoring Category
RepA_N, repUS15	27	1	0	EXP
Rep3, rep29	26	1	0	EXP
Rep3, rep11a	1	0	-	N-EXP
Rep3, rep18b	1	0	-	N-EXP

5. SUPPLEMENTARY DATA – PHENOTYPIC AMR DATA

Determination of Minimum Inhibitory Concentration (MIC) values for the DTU Genomic PT strains was performed on two separate occasions at the National Food Institute, Technical University of Denmark (DTU Food). MIC testing was conducted using the Sensititre EUVSEC3 and EUVSEC2 panels for *E. coli*, the EUST2 panel for *S. aureus*, and the EUVENC panel for *E. faecium* and *E. faecalis* (Trek Diagnostic Systems Ltd, UK).

These MIC values are provided as background information only and are not included in the scoring or evaluation of participant results. Interpretation of MICs was performed using the following epidemiological cut-off values (ECOFFs):

- For *E. coli*, ECOFFs were applied according to the EFSA Manual for Reporting Antimicrobial Resistance 2024.
- For *S. aureus* and *E. faecalis*/*E. faecium*, ECOFFs were from EUCAST website (accessed 04 October 2024).

Species	Strain code	Panel	Class	Antimicrobial	ECOFF	MIC (mg/L)
E. coli	**GPT24-01**	EUVSEC3	Aminoglycoside	Amikacin	**8**	≤ 4
				Gentamicin	**2**	≤ 0.5
			Amphenicol	Chloramphenicol	**16**	≤ 8
			Beta-lactam	Ampicillin	**8**	> 32
				Cefotaxime	**0.25**	> 4
				Ceftazidime	**0.5**	> 8
				Meropenem	**0.12**	= 16
			Folate pathway antagonist	Sulfamethoxazole	**64**	> 512
				Trimethoprim	**2**	> 16
			Macrolide	Azithromycin	**16**	= 32
			Polymyxin	Colistin	**2**	≤ 1
			Quinolone	Ciprofloxacin	**0.06**	> 8
				Nalidixic acid	**8**	> 64
			Tetracycline	Tetracycline	**8**	> 32
				Tigecycline	**0.5**	≤ 0.25
		EUVSEC2	Beta-lactam	Cefepime	**0.12**	> 32
				Cefotaxime	**0.25**	> 64
				Cefotaxime/Clavulanic acid	**0.25**	> 64
				Cefoxitin	**8**	> 64
				Ceftazidime	**0.5**	> 128
				Ceftazidime/clavulanic acid	**0.5**	> 128
				Ertapenem	**0.06**	> 2
				Imipenem	**0.5**	= 4
				Meropenem	**0.12**	> 16
				Temocillin	**16**	= 128
	GPT24-02	EUVSEC3	Aminoglycoside	Amikacin	**8**	≤ 4
				Gentamicin	**2**	≤ 0.5
			Amphenicol	Chloramphenicol	**16**	≤ 8
			Beta-lactam	Ampicillin	**8**	> 32
				Cefotaxime	**0.25**	≤ 0.25
				Ceftazidime	**0.5**	≤ 0.25
				Meropenem	**0.12**	≤ 0.03
			Folate pathway antagonist	Sulfamethoxazole	**64**	≤ 8
				Trimethoprim	**2**	≤ 0.25
			Macrolide	Azithromycin	**16**	≤ 2
			Polymyxin	Colistin	**2**	= 4
			Quinolone	Ciprofloxacin	**0.06**	≤ 0.015
				Nalidixic acid	**8**	≤ 4
				Tetracycline	**8**	≤ 2

Species	Strain code	Panel	Class	Antimicrobial	ECOFF	MIC (mg/L)
			Tetracycline	Tigecycline	**0.5**	≤ 0.25
		EUVSEC2	Beta-lactam	Cefepime	**0.12**	≤ 0.06
				Cefotaxime	**0.25**	≤ 0.25
				Cefotaxime/Clavulanic acid	**0.25**	≤ 0.06
				Cefoxitin	**8**	= 2
				Ceftazidime	**0.5**	≤ 0.25
				Ceftazidime/clavulanic acid	**0.5**	≤ 0.12
				Ertapenem	**0.06**	≤ 0.015
				Imipenem	**0.5**	≤ 0.12
				Meropenem	**0.12**	≤ 0.03
				Temocillin	**16**	= 4
S. aureus	**GPT24-03**	EUST2	Aminoglycoside	Gentamicin	**2**	≤ 0.5
				Kanamycin	**8**	≤ 4
				Streptomycin	**16**	≤ 4
			Amphenicol	Chloramphenicol	**16**	= 8
			Beta-lactam	Cefoxitin	**4**	= 16
				Penicillin	**0.12**	> 1
			Folate pathway antagonist	Sulfamethoxazole	**128**	≤ 64
				Trimethoprim	**2**	≤ 1
			Glycopeptide	Vancomycin	**2**	≤ 1
			Lincosamide	Clindamycin	**0.25**	≤ 0.12
			Macrolide	Erythromycin	**1**	≤ 0.25
			Oxazolidinone	Linezolid	**4**	= 2
			Pleuromutilin	Tiamulin	**2**	= 1
			Pseudomonic acid	Mupirocin	**1**	≤ 0.5
			Quinolone	Ciprofloxacin	**2**	= 0.5
			Rifamycin	Rifampin	**0.03**	≤ 0.015
			Steroid antibacterial	Fusidic acid	**0.5**	≤ 0.25
			Streptogramin	Quinupristin-dalfopristin	**1**	≤ 0.5
			Tetracycline	Tetracycline	**1**	≤ 0.5
	GPT24-04	EUST2	Aminoglycoside	Gentamicin	**2**	≤ 0.5
				Kanamycin	**8**	≤ 4
				Streptomycin	**16**	≤ 4
			Amphenicol	Chloramphenicol	**16**	= 8
			Beta-lactam	Cefoxitin	**4**	= 4
				Penicillin	**0.12**	> 1
			Folate pathway antagonist	Sulfamethoxazole	**128**	≤ 64
				Trimethoprim	**2**	≤ 1
			Glycopeptide	Vancomycin	**2**	≤ 1
			Lincosamide	Clindamycin	**0.25**	≤ 0.12
			Macrolide	Erythromycin	**1**	> 8
			Oxazolidinone	Linezolid	**4**	= 2
			Pleuromutilin	Tiamulin	**2**	≤ 0.5
			Pseudomonic acid	Mupirocin	**1**	≤ 0.5
			Quinolone	Ciprofloxacin	**2**	= 2
			Rifamycin	Rifampin	**0.03**	≤ 0.015
			Steroid antibacterial	Fusidic acid	**0.5**	> 4
			Streptogramin	Quinupristin-dalfopristin	**1**	≤ 0.5
			Tetracycline	Tetracycline	**1**	> 16
E. faecalis	**GPT24-05**	EUVENC	Aminoglycoside	Gentamicin	**64**	≤ 8
			Amphenicol	Chloramphenicol	**32**	= 32
			Beta-lactam	Ampicillin	**4**	= 1
			Glycopeptide	Teicoplanin	**2**	≤ 0.5
				Vancomycin	**4**	= 2
			Lipopeptide	Daptomycin	**4**	= 1
			Macrolide	Erythromycin	**4**	> 128
			Oxazolidinone	Linezolid	**4**	= 8

Species	Strain code	Panel	Class	Antimicrobial	ECOFF	MIC (mg/L)
			Quinolone	Ciprofloxacin	**4**	= 1
			Streptogramin	Quinupristin-dalfopristin	**32**	= 16
			Tetracycline	Tetracycline	**4**	= 128
				Tigecycline	**0.25**	= 0.12
E. faecium	**GPT24-06**	EUVENC	Aminoglycoside	Gentamicin	**32**	≤ 8
			Amphenicol	Chloramphenicol	**32**	≤ 4
			Beta-lactam	Ampicillin	**4**	> 64
			Glycopeptide	Teicoplanin	**2**	≤ 0.5
				Vancomycin	**4**	= 16
			Lipopeptide	Daptomycin	**8**	= 4
			Macrolide	Erythromycin	**4**	= 4
			Oxazolidinone	Linezolid	**4**	= 2
			Quinolone	Ciprofloxacin	**8**	> 16
			Streptogramin	Quinupristin-dalfopristin	**2**	= 4
			Tetracycline	Tetracycline	**4**	= 32
				Tigecycline	**0.25**	= 0.06

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