DTU GENOMIC PROFICIENCY TEST (PT) 2023

“A guide on the interpretation of the submitted data and the scoring system”

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Table of Contents

1. INTRODUCTION

This document is intended for participants of the DTU Genomic Proficiency Test (PT) 2023 (https://www.food.dtu.dk/english/topics/antimicrobial-resistance/eurl-ar/whole-genome-sequencing/genomic-proficiency-test/genomic-proficiency-test-2023), which are members of the EURL-AR and SEQAFRICA networks. It serves as a guide to help understand the evaluation results of individual laboratories.

1.1. Scoring Principles

There is no pass or fail evaluation. The scoring principles in the individual evaluation reports of the participating laboratories is as follows:

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

1.2. Strains and Antimicrobials

The strains and the antimicrobials included in DTU Genomic PT 2023 are presented in **Table 1** and **Table 2**, respectively. Two types of samples were analysed for each strain: DNA isolated by the participants from live cultures (BACT) and pre-prepared DNA by EURL-AR (DNA).

1.3. Required Analyses and Reporting

Participants were requested to perform three types of analyses and report the following results for each strain/type-of-sample combination:

- Multilocus Sequence Typing (MLST).
- Antimicrobial Resistance (AMR) determinants: including AMR mutations and AMR genes associated with the specific panel of antimicrobials for each organism, according to the Genomic PT 2023 protocol (**Table 2**).
- Predicted AMR phenotype: based on the specific panel of antimicrobials for each organism, according to the Genomic PT 2023 protocol (**Table 2**).
- Predicted plasmid replicon types.

Table 1 – Strains included in the DTU Genomic Proficiency Test 2023.

Species	Strain code	Type of sample
Escherichia coli	GENOMIC23-001	BACT/DNA
	GENOMIC23-002	BACT/DNA
Salmonella enterica	GENOMIC23-003	BACT/DNA
	GENOMIC23-004	BACT/DNA
Staphylococcus aureus	GENOMIC23-005	BACT/DNA
	GENOMIC23-006	BACT/DNA

Table 2 – Antimicrobial compounds included in the DTU Genomic Proficiency Test 2023.

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

1.4. Generation of Reference Genomes

To generate the expected results, closed genomes were generated for each strain using hybrid assembly, which served as the reference. Information on the genome size and composition for each strain is presented in **Table 4**. Details on the sequencing and the bioinformatics analysis are provided below:

Long-Read Sequencing

- Platform: Oxford Nanopore Technology (ONT) **MinION**.
- Flowcell: **R10.4.1**.
- Library Prep: 1D Ligation Kit (SQK-LSK114-96).
- Basecalling: Guppy (version 6.3.9).
- Downsampling: SeqKit (version 2.1.0) (Shen et al., 2024, 2016).
- Quality control and trimming: Nanoplot (version 1.43.0), 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.

Short-Read Sequencing

- Platform: Illumina **NovaSeq 6000** (Paired-end 150 bp).
- Library Prep: **Nextera** FLEX Kit.
- Data Conversion: bcl2fastq (version 2.20) to FASTQ files.
- Quality Control:
    - Low-quality reads removed using the Illumina Chastity Filter.
    - PhiX control signal removed with an in-house protocol.
    - Adapter trimming performed for reads longer than 50 bp.
    - Final quality check using FASTQC (version 0.11.8).
- Downsampling: BBmap (version 38.90).
- Quality control and trimming: FastQC (version 0.12.1), MultiQC (version 1.23) and Fastp (version 0.23.4)

Contamination Assessment

Species determination and contamination was 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.

Hybrid Assembly

- A first draft assembly was generated using **Autocycler** (version 0.2.1) (Wick, Ryan R., 2025) 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) and Hybracter (version 0.11.0) were also used to confirm the presence of very small plasmids.

Quality Control of the Assembled Genomes

Quality control of assembled genomes was carried out using QUAST (version 5.2.0), completeness and contamination was evaluated using CheckM2 (version 1.0.2), and BUSCO (version 5.8.2).

Genome Annotation

Annotation was carried out using **Rapid Annotation using Subsystem Technology (RAST)** (Aziz et al., 2008; Overbeek et al., 2014).

Submission of Genomic Data to the European Nucleotide Archive (ENA)

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 3).

Table 3 – Accession numbers of genomic data from the DTU Genomic Proficiency Test 2023 reference strains submitted to ENA.

Organism	Strain	Study Accession	Sample Accession	Accession Number
E. coli	GENOMIC23-001	PRJEB86005	ERS23842145	GCA_965319085
	GENOMIC23-002	PRJEB86005	ERS23842146	GCA_965318735
Salmonella	GENOMIC23-003	PRJEB86005	ERS23842147	GCA_965318675
	GENOMIC23-004	PRJEB86005	ERS23842148	GCA_965318305
S. aureus	GENOMIC23-005	PRJEB86005	ERS23842149	GCA_965327205
	GENOMIC23-006	PRJEB86005	ERS23842150	GCA_965319415

Information on the Genome Composition of each GPT Strain

Table 4 - Genome size and composition for each strain of the DTU Genomic Proficiency Test 2023.

Organism	Strain	Molecule type	Size (bp)	GC%	Circularity
E. coli	GENOMIC23-001	Chromosome	4,760,692	50.8	Circular
		Plasmid_1	275,956	46.1	Circular
		Plasmid_2	94,982	51.4	Circular
		Plasmid_3	39,582	44.2	Circular
		Plasmid_4	33,310	41.8	Circular
		Plasmid_5	2,305	42.4	Circular
	GENOMIC23-002	Chromosome	4,765,148	50.6	Circular
		Plasmid_1	107,802	50.3	Circular
		Plasmid_2	98,254	52.2	Circular
		Plasmid_3	63,799	51.2	Circular
		Plasmid_4	55,209	46.7	Circular
		Plasmid_5	6,078	49.0	Circular
		Plasmid_6	3,272	45.8	Circular
Salmonella	GENOMIC23-003	Chromosome	4,972,906	52.2	Circular
		Plasmid_1	257,870	46.3	Circular
		Plasmid_2	108,635	51.7	Circular
	GENOMIC23-004	Chromosome	4,939,870	52.0	Circular
		Plasmid_1	176,501	51.8	Circular
		Plasmid_2	87,450	51.5	Circular
		Plasmid_3	5,251	49.2	Circular
		Plasmid_4	3,608	43.2	Circular
		Plasmid_5	2,301	51.5	Circular
		Plasmid_6	1,780	56.6	Circular
S. aureus	GENOMIC23-005	Chromosome	2,936,196	32.9	Circular
		Plasmid_1	4,884	31.4	Circular
	GENOMIC23-006	Chromosome	2,747,394	32.8	Circular
		Plasmid_1	29,061	28.6	Circular

1.5. Bioinformatics Tools for MLST, AMR and Plasmid Typing

The tools used for the generation of the expected results for the different types of analyses are presented in

Table 5.

Table 5 – Tools used for the prediction of the expected results in the DTU Genomic Proficiency Test 2023.

Type of analysis	Tool Name, platform	Tool version, Date	Database Version/Date	Thresholds*	Reference
MLST	MLST 2.0, CGE	2.0.9, 2022-05-11	2023-06-19	100% ID, 100% COV for all 7 loci	(Larsen et al., 2012)
AMR	ResFinder, CGE	4.3.3, 2024-03-22	• ResFinder: 2024-03-22 • PointFinder: 2024-03-08	95% ID, 95% COV	(Bortolaia et al., 2020)
	NCBI AMRFinderPlus	3.11.4	v2023-07-13.2	95% ID, 95% COV	(Feldgarden et al., 2019)
Plasmid typing	PlasmidFinder, CGE	2.0.1, 2020-07-01	2023-01-18	95% ID, 90% COV	(Carattoli et al., 2014)
	MOB-suite package, Galaxy	3.0.3	N/A	95% ID, 90% COV	(Robertson and Nash, 2018)

*COV, coverage; ID, Identity

2. MULTILOCUS-SEQUENCE TYPING (MLST)

Table 6 – Expected MLST data.

Organism	Strain	Sequence Type	Allele 1	Allele 2	Allele 3	Allele 4	Allele 5	Allele 6	Allele 7
E. coli	GENOMIC23-001	**4981**	*adk* 10	*fumC* 616	*gyrB* 4	*icd* 8	*mdh* 8	*purA* 13	*recA* 73
	GENOMIC23-002	**5229**	*adk* 43	*fumC* 41	*gyrB* 15	*icd* 18	*mdh* 11	*purA* 7	*recA* 44
Salmonella	GENOMIC23-003	**34**	*aroC* 10	*dnaN* 19	*hemD* 12	*hisD* 9	*purE* 5	*sucA* 9	*thrA* 2
	GENOMIC23-004	**14**	*aroC* 7	*dnaN* 6	*hemD* 8	*hisD* 8	*purE* 7	*sucA* 8	*thrA* 13
S. aureus	GENOMIC23-005	**239**	*arcC* 2	*aroE* 3	*glpF* 1	*gmk* 1	*pta* 4	*tpi* 4	*yqiL* 3
	GENOMIC23-006	**88**	*arcC* 22	*aroE* 1	*glpF* 14	*gmk* 23	*pta* 12	*tpi* 4	*yqiL* 31

Table 7 – Evaluation of the submitted MLST data.

Organism	Strain	Submitted MLST	No of labs		SCORE		Comment
			BACT	DNA	If submitted	If not submitted	
E. coli	GENOMIC23-001	4981	29	30	**1**	0	Expected
		741	1	1	0	N/A	Not expected
	GENOMIC23-002	5229	29	30	**1**	0	Expected
		88	1	1	0	N/A	Not expected
S. enterica	GENOMIC23-003	34	29	30	**1**	0	Expected
		14	1	0	0	N/A	Not expected
		0	1	1	0	N/A	Not expected
	GENOMIC23-004	14	28	29	**1**	0	Expected
		34	1	1	0	N/A	Not expected
		54	1	1	0	N/A	Not expected
S. aureus	GENOMIC23-005	239	27	30	**1**	0	Expected
		0	1	0	0	N/A	Not expected
	GENOMIC23-006	88	27	30	**1**	0	Expected
		0	1	0	0	N/A	Not expected

3. *E. coli* GENOMIC23-001 (BACT/DNA)

3.1. Expected mutations, genes and predicted phenotype, related to AMR

Table 8 – Expected results for *E. coli* - GENOMIC23-001 (BACT/DNA).

Class	Antimicrobial	Pred. R/S	AMR Gene or mutation	Protein name	Protein Accession no	%Identity, %Coverage	Location
Aminoglycosides	AMI	S	None	N/A	N/A	N/A	N/A
	GEN	**R**	*aac(3)-IVa*[1]	AAC(3)-IV or AAC(3)-IVa family aminoglycoside N-acetyltransferase	WP_001199192.1	100, 100	Plasmid 1
			aac(3)-IId	Aminoglycoside N-acetyltransferase AAC(3)-IId	WP_000557454.1	100, 100	Plasmid 2
Amphenicols	CHL	**R**	*cmlA1*	Chloramphenicol efflux MFS transporter CmlA1	WP_000095725.1	100, 100	Plasmid 1
Beta-lactams	AMP	**R**	$bla_{\text{CTX-M-15}}$	Extended-spectrum class A beta-lactamase CTX-M-15	WP_000239590.1	100, 100	Chromosome
			$bla_{\text{TEM-1}}$[2]	Broad-spectrum class A beta-lactamase TEM-1	WP_000027057.1	100, 100	Plasmid 2
	FEP	**R**	$bla_{\text{CTX-M-15}}$	Extended-spectrum class A beta-lactamase CTX-M-15	WP_000239590.1	100, 100	Chromosome
	FOT	**R**	$bla_{\text{CTX-M-15}}$	Extended-spectrum class A beta-lactamase CTX-M-15	WP_000239590.1	100, 100	Chromosome
	FOX	S	None	N/A	N/A	N/A	N/A
	TAZ	**R**	$bla_{\text{CTX-M-15}}$	Extended-spectrum class A beta-lactamase CTX-M-15	WP_000239590.1	100, 100	Chromosome
	ETP	S	None	N/A	N/A	N/A	N/A
	IMI	S	None	N/A	N/A	N/A	N/A
	MERO	S	None	N/A	N/A	N/A	N/A

[1] Depending on the AMR prediction tool used, ResFinder identifies *aac(3)-IV*, while AMRFinderPlus detects *aac(3)-IVa* at the same location. Both are accepted.

[2] Depending on the AMR prediction tool used, ResFinder identifies $bla_{\text{TEM-1B}}$, while AMRFinderPlus detects $bla_{\text{TEM-1}}$ at the same location. Genes $bla_{\text{TEM-1A}}$, $bla_{\text{TEM-1B}}$, $bla_{\text{TEM-1C}}$, and $bla_{\text{TEM-1D}}$ are sequence variants of $bla_{\text{TEM-1}}$, differing by minor nucleotide polymorphisms that do not alter their β-lactamase activity or resistance phenotype. As the DTU Genomic proficiency test evaluates the detection of AMR determinants rather than sequence-level variations, all are accepted under $bla_{\text{TEM-1}}$.

Table 8 – Expected results for *E. coli* - GENOMIC23-001 (BACT/DNA).

Class	Antimi-crobial	Pred. R/S	AMR Gene or mutation	Protein name	Protein Accession no	%Identity, %Coverage	Location
	TRM	S	None	N/A	N/A	N/A	N/A
Folate pathway antagonists	SMX	**R**	*sul3*	Sulphonamide-resistant dihydropteroate synthase Sul3	WP_000034420.1	100, 100	*Two copies:* - Plasmid 1 - Plasmid 2
	TMP	**R**	*dfrA12*	Trimethoprim-resistant dihydrofolate reductase DfrA12	WP_001083725.1	100, 100	Plasmid 1
Macrolides	AZI	**R**	*mef(B)*	Macrolide efflux MFS transporter Mef(B)	WP_032492336.1	100, 100	Plasmid 1
Polymyxins	COL	**R**	*mcr-1.1*	Lipid A phosphoethanolamine transferase MCR-1.1	WP_049589868.1	100, 100	Plasmid 4
Quinolones	CIP	**R**	*gyrA* p.S83L *gyrA* p.D87N	DNA gyrase subunit A GyrA	WP_001281243.1	98.9, 99.7	Chromosome
			parE p.S458A	DNA topoisomerase IV subunit B ParE	WP_033554192.1	99.7, 100	Chromosome
			parC p.S80I	DNA topoisomerase IV subunit A ParC	WP_001281881.1	99.9, 100	Chromosome
	NAL	**R**	*gyrA* p.S83L *gyrA* p.D87N	DNA gyrase subunit A GyrA	WP_001281243.1	98.9, 99.7	Chromosome
			parE p.S458A	DNA topoisomerase IV subunit B ParE	WP_033554192.1	99.7, 100	Chromosome
			parC p.S80I	DNA topoisomerase IV subunit A ParC	WP_001281881.1	99.9, 100	Chromosome
Tetracyclines	TET	**R**	*tet(B)*	Tetracycline efflux MFS transporter Tet(B)	WP_001089068.1	100, 100	Plasmid 1
	TGC	S	None	N/A	N/A	N/A	N/A

3.2. Evaluation of submitted results

3.2.1. AMR mutations

Table 9 – Submitted AMR mutations for *E. coli* - GENOMIC23-001.

Class	Gene	Mutation	No of labs		SCORE		Comment
			BACT	DNA	If submitted	If not submitted	
Quinolones	*gyrA*	D87N	26	27	**1**	0	Expected
		S83L	25	26	**1**	0	Expected
	parC	S80I	27	28	**1**	0	Expected
		S83L	0	1	0	N/A	Not expected
	parE	S458A	25	27	**1**	0	Expected

3.2.2. AMR Genes

Table 10 – Submitted AMR genes for *E. coli* - GENOMIC23-001.

Class	Gene	No of labs		SCORE		Comment
		BACT	DNA	If submitted	If not submitted	
Aminoglycosides	*aac(3)-IId*	23	21	**1**	0	Expected
	aac(3)-IV	19	19	**1**	0	Expected
	aph(3'')-Ib	9	9	0	N/A	Out of scope[3]
	aph(3')-Ia	8	8	0	N/A	Out of scope[3]
	aph(4)-Ia	8	8	0	N/A	Out of scope[3]
	aadA1	8	8	0	N/A	Out of scope[3]
	aadA2	8	8	0	N/A	Out of scope[3]
	aph(6)-Id	7	8	0	N/A	Out of scope[3]
	aac(3)-IVa	7	8	**1**	0	Expected
	aac(3)-IIa	3	3	0	N/A	Not expected
	ant(3'')-Ia	1	2	0	N/A	Out of scope[3]
	aph(6)-Ib	1	0	0	N/A	Not expected
	aac(3)-IIb	1	1	0	N/A	Not expected
	grmA	0	1	0	N/A	Not expected
Amphenicols	*cmlA1*	26	27	**1**	0	Expected
Beta-lactams	$bla_{\text{CTX-M-15}}$	28	31	**1**	0	Expected
	$bla_{\text{TEM-1B}}$	25	26	**1**	0	Expected
	$bla_{\text{TEM-1}}$	2	2	**1**	0	Expected
	$bla_{\text{TEM-15}}$	1	1	0	N/A	Not expected
Folate pathway antagonists	*dfrA12*	27	27	**1**	0	Expected
	sul3	27	26	**1**	0	Expected
	dfrA1	0	1	0	N/A	Not expected
Macrolides	*mef(B)*	27	27	**1**	0	Expected

[3] The AMR gene is present in the genome, but it is related to an AMR phenotype which is out of the scope of the DTU Genomic PT 2023, *i.e.*, it is not in the panel antimicrobials, as presented in **Table 2**. Therefore, it should not have been reported.

Table 10 – Submitted AMR genes for *E. coli* - GENOMIC23-001.

Class	Gene	No of labs		SCORE		Comment
		BACT	DNA	If submitted	If not submitted	
Polymyxins	*mcr-1.1*	28	29	**1**	0	Expected
Tetracyclines	*tet(B)*	29	30	**1**	0	Expected

3.2.3. Predicted AMR phenotype

Table 11 – Submitted predicted AMR phenotype for *E. coli* - GENOMIC23-001.

Class	Antimicrobial	No of labs		SCORE		Comment
		BACT	DNA	If submitted	If not submitted	
Aminoglycosides	Gentamicin	29	30	**1**	0	Expected
Amphenicols	Chloramphenicol	29	30	**1**	0	Expected
Beta-lactams	Ampicillin	28	29	**1**	0	Expected
	Cefepime	27	28	**1**	0	Expected
	Cefotaxime	27	28	**1**	0	Expected
	Ceftazidime	27	28	**1**	0	Expected
Folate pathway antagonists	Trimethoprim	28	28	**1**	0	Expected
	Sulfamethoxazole	27	28	**1**	0	Expected
Macrolides	Azithromycin	25	26	**1**	0	Expected
Polymyxins	Colistin	28	29	**1**	0	Expected
Quinolones	Ciprofloxacin	26	27	**1**	0	Expected
	Nalidixic acid	26	27	**1**	0	Expected
Tetracyclines	Tetracycline	29	30	**1**	0	Expected

4. *E. coli* GENOMIC23-002 (BACT/DNA)

4.1. Expected mutations, genes and predicted phenotype, related to AMR

Table 12 – Expected results for *E. coli* - GENOMIC23-002 (BACT/DNA).

Class	Antimi-crobial	Pred. R/S	AMR Gene or mutation	Protein name	Protein Accession no	%Identity, %Coverage	Location
Aminoglycosides	AMI	S	None	N/A	N/A	N/A	N/A
	GEN	**R**	*aac(3)-IId*	Aminoglycoside N-acetyltransferase AAC(3)-IId	WP_000557454.1	100, 100	Plasmid 2
Amphenicols	CHL	**R**	*cmlA1*	Chloramphenicol efflux MFS transporter CmlA1	WP_000095725.1	100, 100	Plasmid 2
			floR	Chloramphenicol/florfenicol efflux MFS transporter FloR	WP_000214123.1	99.5, 100	Plasmid 2
Beta-lactams	AMP	**R**	*bla*$_{\text{CMY-2}}$	Class C beta-lactamase CMY-2	WP_000976514.1	100, 100	Plasmid 1
			bla$_{\text{OXA-181}}$	OXA-48 family carbapenem-hydrolyzing class D beta-lactamase OXA-181	WP_015060052.1	100, 100	Plasmid 4
			bla$_{\text{TEM-30}}$	Inhibitor-resistant broad-spectrum class A beta-lactamase TEM-30	WP_021561477.1	100, 100	Plasmid 3
			bla$_{\text{TEM-1}}$[4]	Broad-spectrum class A beta-lactamase TEM-1	WP_000027057.1	100, 100	Plasmid 2
	FEP	**R** *or* S[5]	*bla*$_{\text{OXA-181}}$	OXA-48 family carbapenem-hydrolyzing class D beta-lactamase OXA-181	WP_015060052.1	100, 100	Plasmid 4
	FOT	**R**	*bla*$_{\text{CMY-2}}$	Class C beta-lactamase CMY-2	WP_000976514.1	100, 100	Plasmid 1
	FOX	**R**	*bla*$_{\text{CMY-2}}$	Class C beta-lactamase CMY-2	WP_000976514.1	100, 100	Plasmid 1

[4] Depending on the AMR prediction tool used, ResFinder identifies *bla*$_{\text{TEM-1B}}$, while AMRFinderPlus detects *bla*$_{\text{TEM-1}}$ at the same location. Genes *bla*$_{\text{TEM-1A}}$, *bla*$_{\text{TEM-1B}}$, *bla*$_{\text{TEM-1C}}$, and *bla*$_{\text{TEM-1D}}$ are sequence variants of *bla*$_{\text{TEM-1}}$, differing by minor nucleotide polymorphisms that do not alter their β-lactamase activity or resistance phenotype. As the DTU Genomic proficiency test evaluates the detection of AMR determinants rather than sequence-level variations, all are accepted under *bla*$_{\text{TEM-1}}$.

[5] The susceptibility of *bla*$_{\text{OXA-181}}$-producing isolates to cefepime and carbapenems varies according to the literature. Therefore both R and S predicted phenotypes are accepted for cefepime, meropenem, imipenem and ertapenem (Carfora et al., 2022; Ghany et al., 2018; Kidd et al., 2020; Moreira da Silva et al., 2024).

Table 12 – Expected results for *E. coli* - GENOMIC23-002 (BACT/DNA).

Class	Antimi-crobial	Pred. R/S	AMR Gene or mutation	Protein name	Protein Accession no	%Identity, %Coverage	Location
	TAZ	**R**	*bla*$_{CMY-2}$	Class C beta-lactamase CMY-2	WP_000976514.1	100, 100	Plasmid 1
	ETP	**R** *or* S[5]	*bla*$_{OXA-181}$	OXA-48 family carbapenem-hydrolyzing class D beta-lactamase OXA-181	WP_015060052.1	100, 100	Plasmid 4
	IMI	**R** *or* S[5]	*bla*$_{OXA-181}$	OXA-48 family carbapenem-hydrolyzing class D beta-lactamase OXA-181	WP_015060052.1	100, 100	Plasmid 4
	MERO	**R** *or* S[5]	*bla*$_{OXA-181}$	OXA-48 family carbapenem-hydrolyzing class D beta-lactamase OXA-181	WP_015060052.1	100, 100	Plasmid 4
	TRM	**R** *or* S[6]	*bla*$_{OXA-181}$	OXA-48 family carbapenem-hydrolyzing class D beta-lactamase OXA-181	WP_015060052.1	100, 100	Plasmid 4
Folate pathway antagonists	SMX	**R**	*sul2*	Sulfonamide-resistant dihydropteroate synthase Sul2	WP_001043260.1	100, 100	Plasmid 2
			sul3	Sulfonamide-resistant dihydropteroate synthase Sul3	WP_000034420.1	100, 100	Plasmid 2
	TMP	**R**	*dfrA12*	Trimethoprim-resistant dihydrofolate reductase DfrA12	WP_001083725.1	100, 100	Plasmid 2
Macrolides	AZI	S	None	N/A	N/A	N/A	N/A
Polymyxins	COL	**R** *or* S[7]	*pmrB* p.Y358N (optional)[7]	Two-component system sensor histidine kinase PmrB	WP_001300761.1	99.2, 100	Chromosome
Quinolones	CIP	**R**	*gyrA* p.S83L *gyrA* p.D87N	DNA gyrase subunit A GyrA	WP_001281243.1	99.0, 99.7	Chromosome
			parC p.S80I	DNA topoisomerase IV subunit A ParC	WP_001281881.1	99.9, 100	Chromosome

[6] The hydrolytic profile of the OXA-48 family carbapenem-hydrolyzing class D beta-lactamase OXA-181 includes hydrolytic activity against temocillin, according to the Beta-Lactamase Database (BLDB) – See **Figure 1**. Predicted resistance to temocillin is not clearly outputted by neither ResFinder nor AMRFinderPlus, therefore both R and S phenotypes are accepted for temocillin.

[7] The Y358N mutation in the PmrB protein of *E. coli* has been identified in both colistin-resistant and colistin-susceptible isolates, suggesting that this specific mutation alone may not be sufficient to confer resistance to colistin (Choi et al., 2020; Luo et al., 2017). Moreover, this mutation is outputted by AMRFinderPlus but not ResFinder. Therefore, both colistin-resistant and colistin-susceptible phenotypes are accepted.

Table 12 – Expected results for *E. coli* - GENOMIC23-002 (BACT/DNA).

Class	Antimi-crobial	Pred. R/S	AMR Gene or mutation	Protein name	Protein Accession no	%Identity, %Coverage	Location
	NAL	**R**	*gyrA* p.S83L *gyrA* p.D87N	DNA gyrase subunit A GyrA	WP_001281243.1	99.0, 99.7	Chromosome
			parC p.S80I	DNA topoisomerase IV subunit A ParC	WP_001281881.1	99.9, 100	Chromosome
Tetracyclines	TET	**R**	*tet(A)*	Tetracycline efflux MFS transporter Tet(A)	WP_000804064.1	99.8, 100	Plasmid 2
			tet(M) (optional)[8]	Tetracycline resistance ribosomal protection protein Tet(M)	WP_063856397.1	98.1, 100	Plasmid 2
	TGC	S	None	N/A	N/A	N/A	N/A

[8] Multiple studies in tetracycline resistance in *E. coli* have identified the *tet(M)* gene among several AMR determinants; however, the available data indicate that while *tet(M)* contributes to the resistance phenotype, its presence alone is not sufficient to cause high-level tetracycline resistance, and therefore, reporting it is optional (Bryan et al., 2004; Hu et al., 2013; Jones et al., 2006; Tuckman et al., 2007).

4.2. Evaluation of submitted results

4.2.1. AMR mutations

Table 13 – Submitted AMR mutations for *E. coli* - GENOMIC23-002.

Class	Gene	Mutation	No of labs		SCORE		Comment
			BACT	DNA	If submitted	If not submitted	
Quinolones	*gyrA*	D87N	27	28	**1**	0	Expected
		S83L	27	28	**1**	0	Expected
	parC	S80I	27	28	**1**	0	Expected
Polymyxins	*pmrB*	Y358N	1	2	**1**	**Blank**	Accepted[7]

4.2.2. AMR Genes

Table 14 – Submitted AMR genes for *E. coli* - GENOMIC23-002.

Class	Gene	No of labs		SCORE		Comment
		BACT	DNA	If submitted	If not submitted	
Aminoglycosides	*aac(3)-IId*	24	26	**1**	0	Expected
	aadA2	8	8	0	N/A	Out of scope[9]
	aadA1	6	6	0	N/A	Out of scope[9]
	ant(3'')-Ia	3	3	0	N/A	Out of scope[9]
	aac(3)-IIa	3	3	0	N/A	Not expected
	aadA17	1	1	0	N/A	Out of scope[9]
Amphenicols	*cmlA1*	25	25	**1**	0	Expected
	floR	24	25	**1**	0	Expected
Beta-lactams	$bla_{\text{CMY-2}}$	28	30	**1**	0	Expected
	$bla_{\text{OXA-181}}$	23	30	**1**	0	Expected
	$bla_{\text{TEM-30}}$	18	14	**1**	0	Expected
	$bla_{\text{TEM-1B}}$	7	12	**1**	0	Expected
	$bla_{\text{TEM-207}}$	2	2	0	N/A	Not expected
	$bla_{\text{TEM-1}}$	1	1	**1**	0	Expected
	$bla_{\text{TEM-104}}$	1	1	0	N/A	Not expected
	$bla_{\text{TEM-176}}$	1	1	0	N/A	Not expected
	$bla_{\text{TEM-198}}$	1	1	0	N/A	Not expected
	$bla_{\text{TEM-217}}$	1	1	0	N/A	Not expected
	$bla_{\text{TEM-230}}$	1	1	0	N/A	Not expected
	$bla_{\text{TEM-234}}$	1	1	0	N/A	Not expected
	$bla_{\text{TEM-70}}$	1	1	0	N/A	Not expected
Folate pathway antagonists	*dfrA12*	28	28	**1**	0	Expected
	sul2	27	27	**1**	0	Expected
	sul3	24	24	**1**	0	Expected

[9] The AMR gene is present in the genome, but it is related to an AMR phenotype which is out of the scope of the DTU Genomic PT 2023, *i.e.*, it is not in the panel antimicrobials, as presented in **Table 2**. Therefore, it should not have been reported.

Table 14 – Submitted AMR genes for *E. coli* - GENOMIC23-002.

Class	Gene	No of labs		SCORE		Comment
		BACT	DNA	If submitted	If not submitted	
Tetracyclines	*tet(A)*	26	27	**1**	0	Expected
	tet(M)	16	14	**1**	**Blank**	Accepted[8]

4.2.3. Predicted AMR phenotype

Table 15 – Submitted predicted AMR phenotype for *E. coli* - GENOMIC23-002.

Class	Antimicrobial	No of labs		SCORE		Comment
		BACT	DNA	If submitted	If not submitted	
Aminoglycosides	Gentamicin	29	30	**1**	0	Expected
Amphenicols	Chloramphenicol	29	28	**1**	0	Expected
Beta-lactams	Ampicillin	30	29	**1**	0	Expected
	Cefotaxime	28	28	**1**	0	Expected
	Ceftazidime	28	27	**1**	0	Expected
	Cefoxitin	27	28	**1**	0	Expected
	Cefepime	23	28	**1**	**Blank**	Accepted[5]
	Meropenem	21	27	**1**	**Blank**	Accepted[5]
	Ertapenem	19	25	**1**	**Blank**	Accepted[5]
	Imipenem	19	25	**1**	**Blank**	Accepted[5]
	Temocillin	2	4	**1**	**Blank**	Accepted[6]
Folate pathway antagonists	Trimethoprim	29	28	**1**	0	Expected
	Sulfamethoxazole	28	28	**1**	0	Expected
Polymyxins	Colistin	1	2	**1**	**Blank**	Accepted[7]
Quinolones	Ciprofloxacin	26	26	**1**	0	Expected
	Nalidixic acid	26	27	**1**	0	Expected
Tetracyclines	Tetracycline	29	29	**1**	0	Expected
	Tigecycline	1	1	0	N/A	Not expected

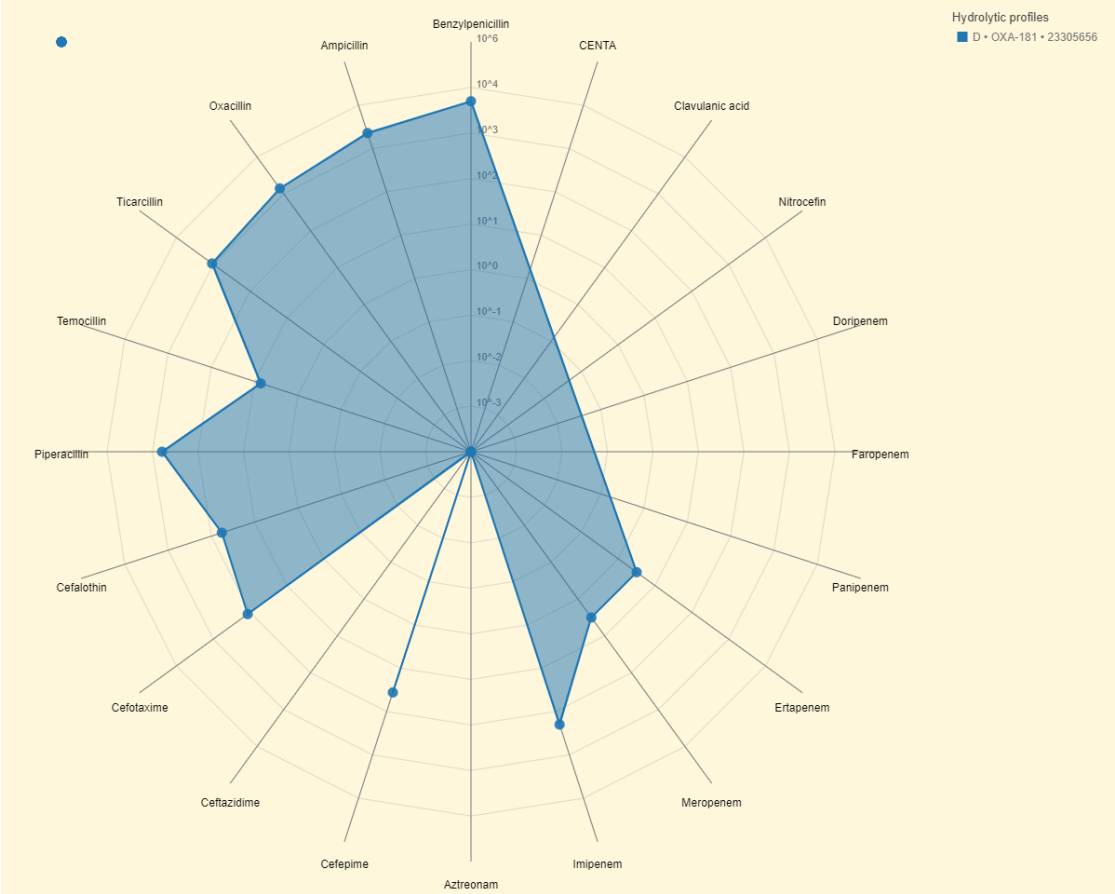

***Figure 1**. Hydrolytic profile of the OXA-48 family carbapenem-hydrolysing class D beta-lactamase OXA-181. Retrieved from Beta-Lactamase Database (BLDB) in July 2023 (Naas et al., 2017).*

5. *S. enterica* GENOMIC23-003 (BACT/DNA)

5.1. Expected mutations, genes and predicted phenotype, related to AMR

Table 16 – Expected results for *S. enterica* - GENOMIC23-003 (BACT/DNA).

Class	Antimi-crobial	Pred. R/S	AMR Gene or mutation	Protein name	Protein Accession no	%Identity, %Coverage	Location
Aminoglycosides	AMI	S	None	N/A	N/A	N/A	N/A
	GEN	**R**	*aac(3)-IId*	Aminoglycoside N-acetyltransferase AAC(3)-IId	WP_000557454.1	100, 100	*Two copies:* - Plasmid 1 - Plasmid 2
Amphenicols	CHL	**R**	*cmlA1*	Chloramphenicol efflux MFS transporter CmlA1	WP_000095725.1	100, 100	Plasmid 2
Beta-lactams	AMP	**R**	*bla*$_{\text{TEM-1}}$[10]	Broad-spectrum class A beta-lactamase TEM-1	WP_000027057.1	100, 100	*Two copies:* Chromosome Plasmid 1
			bla$_{\text{SHV-12}}$	Extended-spectrum class A beta-lactamase SHV-12	WP_002904004.1	100, 100	Plasmid 1
	FEP	**R**	*bla*$_{\text{SHV-12}}$	Extended-spectrum class A beta-lactamase SHV-12	WP_002904004.1	100, 100	Plasmid 1
	FOT	**R**	*bla*$_{\text{SHV-12}}$	Extended-spectrum class A beta-lactamase SHV-12	WP_002904004.1	100, 100	Plasmid 1
	FOX	S	None	N/A	N/A	N/A	N/A
	TAZ	**R**	*bla*$_{\text{SHV-12}}$	Extended-spectrum class A beta-lactamase SHV-12	WP_002904004.1	100, 100	Plasmid 1
	ETP	S	None	N/A	N/A	N/A	N/A

[10] Depending on the AMR prediction tool used, ResFinder identifies *bla*$_{\text{TEM-1B}}$, while AMRFinderPlus detects *bla*$_{\text{TEM-1}}$, both perfect hits at the same location. Genes *bla*$_{\text{TEM-1A}}$, *bla*$_{\text{TEM-1B}}$, *bla*$_{\text{TEM-1C}}$, and *bla*$_{\text{TEM-1D}}$ are sequence variants of *bla*$_{\text{TEM-1}}$, differing by minor nucleotide polymorphisms that do not alter their β-lactamase activity or resistance phenotype. As the DTU Genomic proficiency test evaluates the detection of AMR determinants rather than sequence-level variations, all are accepted under *bla*$_{\text{TEM-1}}$.

Table 16 – Expected results for *S. enterica* - GENOMIC23-003 (BACT/DNA).

Class	Antimi-crobial	Pred. R/S	AMR Gene or mutation	Protein name	Protein Accession no	%Identity, %Coverage	Location
	IMI	S	None	N/A	N/A	N/A	N/A
	MERO	S	None	N/A	N/A	N/A	N/A
	TRM	S	None	N/A	N/A	N/A	N/A
Folate pathway antagonists	SMX	**R**	*sul2*	Sulfonamide-resistant dihydropteroate synthase Sul2	*Copy 1:* WP_001043265.1 *Copy 2:* WP_001043260.1	*Copy 1:* 100, 100 *Copy 2:* 100, 100	*Copy 1:* Chromosome *Copy 2:* Plasmid 2
	TMP	**R**	*dfrA12*	Trimethoprim-resistant dihydrofolate reductase DfrA12	WP_001083725.1	100, 100	Plasmid 2
Macrolides	AZI	S	None	N/A	N/A	N/A	N/A
Polymyxins	COL	S	None	N/A	N/A	N/A	N/A
Quinolones	CIP	S	None	N/A	N/A	N/A	N/A
	NAL	S	None	N/A	N/A	N/A	N/A
Tetracyclines	TET	S *or* **R**	*tet(M)* (optional)[11]	Tetracycline resistance ribosomal protection protein Tet(M)	WP_063856397.1	98.1, 100	Plasmid 2
	TGC	S	None	N/A	N/A	N/A	N/A

[11] The scientific literature suggests that the tetracycline resistance ribosomal protection protein Tet(M) may be a functional resistance determinant in *S. enterica*; however, there is a lack of robust evidence of whether its presence alone is sufficient to cause high-level tetracycline resistance(McDermott et al., 2016; Russo et al., 2022). Therefore, reporting of the *tet(M)* gene is optional and both tetracycline-susceptible and tetracycline-resistant phenotypes are accepted.

5.2. Evaluation of submitted results

5.2.1. AMR mutations

Table 17 – Submitted AMR mutations for *S. enterica* - GENOMIC23-003.

Class	Gene	Mutation	No of labs		SCORE		Comment
			BACT	DNA	If submitted	If not submitted	
Quinolones	*parC*	A620T	1	1	0	N/A	Out of scope[12]
		L40P	1	1	0	N/A	Not expected
		N395S	1	1	0	N/A	Out of scope[12]
		S469A	1	1	0	N/A	Out of scope[12]
		T255S	1	1	0	N/A	Out of scope[12]
Macrolides	*acrB*	F28L	1	1	0	N/A	Out of scope[12]

5.2.2. AMR Genes

Table 18 – Submitted AMR genes for *S. enterica* - GENOMIC23-003.

Class	Gene	No of labs		SCORE		Comment
		BACT	DNA	If submitted	If not submitted	
Aminoglycosides	*aac(3)-IId*	23	24	**1**	0	Expected
	aac(6')-Iaa	22	22	**Blank**	N/A	Blanked[13]
	aph(3'')-Ib	9	9	0	N/A	Out of scope[14]
	aph(6)-Id	9	9	0	N/A	Out of scope[14]
	aadA2	7	8	0	N/A	Out of scope[14]
	aadA1	5	5	0	N/A	Out of scope[14]
	aac(3)-IIa	4	4	0	N/A	Not expected
	ant(3'')-Ia	2	2	0	N/A	Not expected
	aac(6')-IIa	0	1	0	N/A	Not expected
Amphenicols	*cmlA1*	26	27	**1**	0	Expected
Beta-lactams	bla_{SHV-12}	28	30	**1**	0	Expected
	bla_{TEM-1B}	25	25	**1**	0	Expected
	bla_{TEM-1}	2	2	**1**	0	Expected

[12] The reported mutation is present in the genome, but it is not known to be related to AMR; therefore, it is out of the scope of the DTU Genomic proficiency test and should not have been reported.

[13] This gene is detected by ResFinder but not by AMRFinderPlus. According to ResFinder, it is classified as a cryptic gene in *Salmonella* and does not confer resistance to amikacin. Since it is not associated with a resistant phenotype, it should not have been reported. However, the ResFinder output can be misleading, as the gene's cryptic nature is only noted in a small annotation and does not appear in the phenotype table. As a result, reporting this gene is not penalized.

[14] The AMR gene is present in the genome, but it is related to an AMR phenotype which is out of the scope of the DTU Genomic PT 2023, *i.e.*, it is not in the panel antimicrobials, as presented in **Table 2**. Therefore, it should not have been reported.

Table 18 – Submitted AMR genes for *S. enterica* - GENOMIC23-003.

Class	Gene	No of labs		SCORE		Comment
		BACT	DNA	If submitted	If not submitted	
	bla$_{SHV-1}$	1	0	0	N/A	Not expected
Folate pathway antagonists	*sul2*	27	24	**1**	0	Expected
	dfrA12	26	27	**1**	0	Expected
	dfrA1	0	1	0	N/A	Not expected
Tetracyclines	*tet(M)*	15	17	**1**	**Blank**	Accepted[11]

5.2.3. Predicted AMR phenotype

Table 19 – Submitted predicted AMR phenotype for *S. enterica* - GENOMIC23-003.

Class	Predicted R phenotype to antimicrobial	No of labs		SCORE		Comment
		BACT	DNA	If submitted	If not submitted	
Aminoglycosides	Gentamicin	25	28	**1**	0	Expected
	Amikacin	19	20	0	N/A	Not expected
Amphenicols	Chloramphenicol	27	28	**1**	0	Expected
Beta-lactams	Ampicillin	29	30	**1**	0	Expected
	Cefepime	27	28	**1**	0	Expected
	Cefotaxime	27	27	**1**	0	Expected
	Ceftazidime	26	27	**1**	0	Expected
	Cefoxitin	1	1	0	N/A	Not expected
Folate pathway antagonists	Trimethoprim	25	26	**1**	0	Expected
	Sulfamethoxazole	22	23	**1**	0	Expected
Macrolides	Azithromycin	1	0	0	N/A	Not expected
Polymyxins	Colistin	1	1	0	N/A	Not expected
Quinolones	Ciprofloxacin	1	0	0	N/A	Not expected
Tetracyclines	Tetracycline	16	17	**1**	**Blank**	Accepted[11]

6. *S. enterica* GENOMIC23-004 (BACT/DNA)

6.1. Expected mutations, genes and predicted phenotype, related to AMR

Table 20 – Expected results for *S. enterica* - GENOMIC23-004 (BACT/DNA).

Class	Antimi-crobial	Pred. R/S	AMR Gene or mutation	Protein name	Protein Accession no	%Identity, %Coverage	Location
Aminoglycosides	AMI	**R**	*aac(6')-Ib-cr5*[15]	Fluoroquinolone-acetylating aminoglycoside 6'-N-acetyltransferase AAC(6')-Ib-cr5	WP_063840321.1	100, 100	Plasmid 1
			aac(6')-Ib	AAC(6')-Ib family aminoglycoside 6'-N-acetyltransferase	WP_063840280.1	100, 100	Plasmid 1
			armA	16S rRNA (guanine(1405)-N(7))-methyltransferase ArmA	WP_000359986.1	100, 100	Plasmid 2
	GEN	**R**	*aac(3)-IIe*[15]	Aminoglycoside N-acetyltransferase AAC(3)-IIe	WP_000557452.1	100, 100	Plasmid 1
			aac(3)-IId	Aminoglycoside N-acetyltransferase AAC(3)-IId	WP_000557454.1	100, 100	Plasmid 2
			armA	16S rRNA (guanine(1405)-N(7))-methyltransferase ArmA	WP_000359986.1	100, 100	Plasmid 2
Amphenicols	CHL	**R**	*cmlA5*[15]	Chloramphenicol efflux MFS transporter CmlA5	WP_012300772.1	100, 100	Plasmid 1
Beta-lactams	AMP	**R**	$bla_{\text{CTX-M-15}}$	Extended-spectrum class A beta-lactamase CTX-M-15	WP_000239590.1	100, 100	Plasmid 1
			$bla_{\text{NDM-1}}$	Subclass B1 metallo-beta-lactamase NDM-1	WP_004201164.1	100, 100	Plasmid 2
			$bla_{\text{OXA-1}}$	Oxacillin-hydrolyzing class D beta-lactamase OXA-1	WP_001334766.1	100, 100	Plasmid 1
			$bla_{\text{OXA-9}}$	Oxacillin-hydrolyzing class D beta-lactamase OXA-9	WP_000722315.1	100, 100	Plasmid 1

[15] Depending on the AMR prediction tool used, different variant names may be assigned to the same resistance gene at the same genomic location. ResFinder identifies *aac(6')-Ib-cr*, while AMRFinderPlus reports it as *aac(6')-Ib-cr5*; similarly, ResFinder detects *aac(3)-IIa*, whereas AMRFinderPlus identifies it as *aac(3)-IIe*; and for *cmlA1*, ResFinder's annotation corresponds to *cmlA5* in AMRFinderPlus, all as perfect hits. Additionally, ResFinder distinguishes between $bla_{\text{TEM-1A}}$ in plasmid_1 and $bla_{\text{TEM-1B}}$ in plasmid_2, whereas AMRFinderPlus reports both as $bla_{\text{TEM-1}}$, at the same respective locations. All the above genes are accepted. Genes $bla_{\text{TEM-1A}}$, $bla_{\text{TEM-1B}}$, $bla_{\text{TEM-1C}}$, and $bla_{\text{TEM-1D}}$ are sequence variants of $bla_{\text{TEM-1}}$, differing by minor nucleotide polymorphisms that do not alter their β-lactamase activity or resistance phenotype. As the DTU Genomic proficiency test evaluates the detection of AMR determinants rather than sequence-level variations, all are accepted under $bla_{\text{TEM-1}}$.

Table 20 – Expected results for *S. enterica* - GENOMIC23-004 (BACT/DNA).

Class	Antimi-crobial	Pred. R/S	AMR Gene or mutation	Protein name	Protein Accession no	%Identity, %Coverage	Location
			bla$_{OXA-10}$	Oxacillin-hydrolyzing class D beta-lactamase OXA-10	WP_000846390.1	100, 100	Plasmid 1
			bla$_{TEM-1}$[15]	Broad-spectrum class A beta-lactamase TEM-1	WP_000027057.1	100, 100	*Two copies:* - Plasmid 1 - Plasmid 2
	FEP	**R**	*bla*$_{CTX-M-15}$	Extended-spectrum class A beta-lactamase CTX-M-15	WP_000239590.1	100, 100	Plasmid 1
			bla$_{OXA-1}$	Oxacillin-hydrolyzing class D beta-lactamase OXA-1	WP_001334766.1	100, 100	Plasmid 1
			bla$_{NDM-1}$	Subclass B1 metallo-beta-lactamase NDM-1	WP_004201164.1	100, 100	Plasmid 2
	FOT	**R**	*bla*$_{CTX-M-15}$	Extended-spectrum class A beta-lactamase CTX-M-15	WP_000239590.1	100, 100	Plasmid 1
			bla$_{NDM-1}$	Subclass B1 metallo-beta-lactamase NDM-1	WP_004201164.1	100, 100	Plasmid 2
	FOX	**R**	*bla*$_{NDM-1}$	Subclass B1 metallo-beta-lactamase NDM-1	WP_004201164.1	100, 100	Plasmid 2
	TAZ	**R**	*bla*$_{CTX-M-15}$	Extended-spectrum class A beta-lactamase CTX-M-15	WP_000239590.1	100, 100	Plasmid 1
			bla$_{NDM-1}$	Subclass B1 metallo-beta-lactamase NDM-1	WP_004201164.1	100, 100	Plasmid 2
	ETP	**R**	*bla*$_{NDM-1}$	Subclass B1 metallo-beta-lactamase NDM-1	WP_004201164.1	100, 100	Plasmid 2
	IMI	**R**	*bla*$_{NDM-1}$	Subclass B1 metallo-beta-lactamase NDM-1	WP_004201164.1	100, 100	Plasmid 2
	MERO	**R**	*bla*$_{NDM-1}$	Subclass B1 metallo-beta-lactamase NDM-1	WP_004201164.1	100, 100	Plasmid 2
	TRM	**R**	*bla*$_{NDM-1}$	Subclass B1 metallo-beta-lactamase NDM-1	WP_004201164.1	100, 100	Plasmid 2
Folate pathway antagonists	SMX	**R**	*sul1*	Sulfonamide-resistant dihydropteroate synthase Sul1	WP_000259031.1	100, 100	*Two copies:* - Plasmid 1 - Plasmid 2
	TMP	S	None	N/A	N/A	N/A	N/A
Macrolides	AZI	**R**	*mph(A)*	Mph(A) family macrolide 2'-phosphotransferase	WP_000219391.1	99.7, 100	Plasmid 1

Table 20 – Expected results for *S. enterica* - GENOMIC23-004 (BACT/DNA).

Class	Antimi-crobial	Pred. R/S	AMR Gene or mutation	Protein name	Protein Accession no	%Identity, %Coverage	Location
			msr(E)	ABC-F type ribosomal protection protein Msr(E)	WP_000052512.1	100, 100	Plasmid 2
			mph(E) (optional)[16]	Mph(E) family macrolide 2'-phosphotransferase	WP_000155092.1	100, 100	Plasmid 2
Polymyxins	COL	S	None	N/A	N/A	N/A	N/A
Quinolones	CIP	**R**	*aac(6')-Ib-cr5*[15]	Fluoroquinolone-acetylating aminoglycoside 6'-N-acetyltransferase AAC(6')-Ib-cr5	WP_063840321.1	100, 100	Plasmid 1
			gyrA p.S83Y, *gyrA* p.D87G	DNA gyrase subunit A GyrA	WP_001281271.1	99.8, 100	Chromosome
			parC p.T57S, *parC* p.S80I	DNA topoisomerase IV subunit A ParC	WP_001281910.1	99.5, 100	Chromosome
	NAL	**R**	*gyrA* p.S83Y, *gyrA* p.D87G	DNA gyrase subunit A GyrA	WP_001281271.1	99.8, 100	Chromosome
			parC p.T57S, *parC* p.S80I	DNA topoisomerase IV subunit A ParC	WP_001281910.1	99.5, 100	Chromosome
Tetracyclines	TET	S	None	N/A	N/A	N/A	N/A
	TGC	S	None	N/A	N/A	N/A	N/A

[16] In ResFinder, the gene *mph(E)* is linked only to the phenotype "erythromycin," whereas AMRFinderPlus categorizes it under the broader term "macrolides," creating ambiguity. Ge et al. (2023) reported six *Salmonella* isolates harboring *mph(E)* co-located with *msr(E)* on plasmids, referred to as *mph(E)-msr(E)*, with azithromycin MICs of ≥64 mg/L (ECOFF=16), indicating resistance (Ge et al., 2023). In the *Salmonella* isolate GENOMIC23-004, *mph(E)* and *msr(E)* are similarly co-located, with *mph(E)* encoding a macrolide phosphotransferase that chemically modifies macrolides and *msr(E)* encoding a ribosomal protection protein. These distinct mechanisms seem to contribute to azithromycin resistance. Therefore, it should be scientifically valid to associate *mph(E)* with azithromycin resistance. However, due to the discrepancies above, participants who omitted *mph(E)* in their submission, will not be penalized (scores blanked).

6.2. Evaluation of submitted results

6.2.1. AMR mutations

Table 21 – Submitted AMR mutations for *S. enterica* - GENOMIC23-004.

Class	Gene	Mutation	No of labs		SCORE		Comment
			BACT	DNA	If submitted	If not submitted	
Quinolones	*gyrA*	S83Y	27	27	**1**	0	Expected
		D87G	26	26	**1**	0	Expected
		D78G	1	1	0	N/A	Not expected
	parC	S80I	26	27	**1**	0	Expected
		T57S	24	25	**1**	0	Expected

6.2.2. AMR Genes

Table 22 – Submitted AMR genes for *S. enterica* - GENOMIC23-004.

Class	Gene	No of labs		SCORE		Comment
		BACT	DNA	If submitted	If not submitted	
Aminoglycosides	*armA*	26	27	**1**	0	Expected
	aac(3)-IId	18	21	**1**	0	Expected
	aac(6')-Iaa	16	18	**Blank**	**Blank**	Blanked[13]
	aac(3)-IIa	14	16	**1**	0	Expected
	aac(6')-Ib	8	8	**1**	0	Expected
	ant(3'')-Ia	6	5	0	N/A	Out of scope[17]
	aadA1	6	5	0	N/A	Out of scope[17]
	aac(3)-IIe	2	3	**1**	0	Expected
	aac(6')-Ib3	2	3	**1**	**Blank**	Accepted[18]
	aac(6')-IIa	0	1	0	N/A	Not expected
Aminoglycoside, Quinolone	*aac(6')-Ib-cr*	16	18	**1**	0	Expected
	aac(6')Ib-cr	1	1	0	N/A	Not expected
Amphenicol	*cmlA1*	24	25	**1**	0	Expected
	catB3	6	11	0	N/A	Not expected[19]
	cmlA5	1	2	**1**	0	Expected

[17] The AMR gene is present in the genome, but it is related to an AMR phenotype which is out of the scope of the DTU Genomic PT 2023, *i.e.*, it is not in the panel antimicrobials, as presented in **Table 2**. Therefore, it should not have been reported.

[18] This gene is accepted as it is a variant of gene *aac(6')-Ib*, which is expected.

[19] A hit for the chloramphenicol resistance gene *catB3* gene was identified in the reference genome, on plasmid_1, position 117,695 base pairs (bp), with 70% coverage and 100% identity at protein level (reference gene: AJ009818.1; protein: WP_000186237.1). The reference *catB3* gene (630 bp) aligns perfectly with the corresponding plasmid_1 region from base 1 to 442, but multiple insertions and deletions downstream disrupt the reading frame, altering the amino acid sequence. A stop codon at position 563 further truncates the protein, making its functionality uncertain.

Table 22 – Submitted AMR genes for *S. enterica* - GENOMIC23-004.

Class	Gene	No of labs		SCORE		Comment
		BACT	DNA	If submitted	If not submitted	
Beta-lactam	*bla*$_{\text{CTX-M-15}}$	27	29	**1**	0	Expected
	bla$_{\text{NDM-1}}$	27	29	**1**	0	Expected
	bla$_{\text{OXA-10}}$	27	28	**1**	0	Expected
	bla$_{\text{OXA-9}}$	25	25	**1**	0	Expected
	bla$_{\text{OXA-1}}$	24	26	**1**	0	Expected
	bla$_{\text{TEM-1B}}$	11	9	**1**	0	Expected
	bla$_{\text{DHA-1}}$	8	9	0	N/A	Not expected[20]
	bla$_{\text{TEM-1A}}$	6	7	**1**	0	Expected
	bla$_{\text{DHA-24}}$	4	4	0	N/A	Not expected
	bla$_{\text{DHA-7}}$	4	5	0	N/A	Not expected
	bla$_{\text{TEM-1}}$	3	4	**1**	0	Expected
	bla$_{\text{SHV-12}}$	1	1	0	N/A	Not expected
	bla$_{\text{TEM-122}}$	1	0	0	N/A	Not expected
	bla$_{\text{TEM-124}}$	1	0	0	N/A	Not expected
	bla$_{\text{TEM-135}}$	1	0	0	N/A	Not expected
	bla$_{\text{TEM-141}}$	1	0	0	N/A	Not expected
	bla$_{\text{TEM-168}}$	1	1	0	N/A	Not expected
	bla$_{\text{TEM-1C}}$	1	1	**1**	0	Expected
	bla$_{\text{TEM-209}}$	1	0	0	N/A	Not expected
	bla$_{\text{TEM-29}}$	1	0	0	N/A	Not expected
	bla$_{\text{TEM-55}}$	1	0	0	N/A	Not expected
	bla$_{\text{TEM-57}}$	1	0	0	N/A	Not expected
Folate pathway antagonist	*sul1*	25	29	**1**	0	Expected
	sul2	6	6	0	N/A	Not expected
	dfrA12	1	1	0	N/A	Not expected
Macrolide	*mph(A)*	23	25	**1**	0	Expected
	mph(E)	11	12	**1**	**Blank**	Accepted[16]
Macrolide, Streptogramin B	*msr(E)*	22	25	**1**	0	Expected
Tetracycline	*tet(M)*	1	0	0	N/A	Not expected

[20] A hit for *bla*$_{\text{DHA}}$ (or *bla*$_{\text{DHA-1}}$, depending on the AMR tool used. AMRFinderPlus outputs *bla*$_{\text{DHA}}$ and ResFinder *bla*$_{\text{DHA-1}}$) was identified in the reference genome with 70% coverage and 100% identity at protein level. The hit is located on plasmid 2, position 42,215 base pairs. The reference protein (WP_004236386.1, 379 amino acids) and gene (JN638038, 1,140 base pairs) align perfectly up to base pair 795. However, between base pairs 796 and 1,025, multiple indels disrupt the reading frame, resulting in an altered amino acid sequence and a truncated protein due to a stop codon at position 1,026 of the reference gene. It cannot be predicted with certainty whether the resulting protein is functional.

6.2.3. Predicted AMR phenotype

Table 23 – Submitted predicted AMR phenotype for *S. enterica* - GENOMIC23-004.

Class	Predicted R phenotype to antimicrobial	No of labs		SCORE		Comment
		BACT	DNA	If submitted	If not submitted	
Aminoglycoside	Gentamicin	27	30	**1**	0	Expected
	Amikacin	26	29	**1**	0	Expected
Amphenicol	Chloramphenicol	29	30	**1**	0	Expected
Beta-lactam	Ampicillin	29	30	**1**	0	Expected
	Cefepime	27	28	**1**	0	Expected
	Cefotaxime	27	28	**1**	0	Expected
	Ceftazidime	27	28	**1**	0	Expected
	Cefoxitin	26	28	**1**	0	Expected
	Ertapenem	26	27	**1**	0	Expected
	Meropenem	26	28	**1**	0	Expected
	Imipenem	25	26	**1**	0	Expected
	Temocillin	23	24	**1**	0	Expected
Folate pathway antagonist	Sulfamethoxazole	24	26	**1**	0	Expected
	Trimethoprim	0	1	0	N/A	Not expected
Macrolide	Azithromycin	25	26	**1**	0	Expected
Quinolone	Ciprofloxacin	27	28	**1**	0	Expected
	Nalidixic acid	25	26	**1**	0	Expected
Tetracycline	Tetracycline	1	1	0	N/A	Not expected

7. *S. aureus* GENOMIC23-005 (BACT/DNA)

7.1. Expected mutations, genes and predicted phenotype, related to AMR

Table 24 – Expected results for *S. aureus* - GENOMIC23-005 (BACT/DNA).

Class	Antimi-crobial	Pred. R/S	AMR Gene or mutation	Protein name	Protein Accession no	%Identity, %Coverage	Location
Aminoglycosides	GEN	**R**	*aac(6')-Ie/aph(2'')-Ia*[21]	Bifunctional aminoglycoside N-acetyltransferase AAC(6')-Ie/aminoglycoside O-phosphotransferase APH(2'')-Ia	WP_001028144.1	100, 100	Chromosome
	KAN	**R**	*aadD1* (optional)[22]	Aminoglycoside O-nucleotidyltransferase ANT(4')-Ia	WP_001795128.1	100, 100	Chromosome
			aac(6')-Ie/aph(2'')-Ia[21]	Bifunctional aminoglycoside N-acetyltransferase AAC(6')-Ie/aminoglycoside O-phosphotransferase APH(2'')-Ia	WP_001028144.1	100, 100	Chromosome
	STR	**R**	*aac(6')-Ie/aph(2'')-Ia*[21]	Bifunctional aminoglycoside N-acetyltransferase AAC(6')-Ie/aminoglycoside O-phosphotransferase APH(2'')-Ia	WP_001028144.1	100, 100	Chromosome

[21] Gene variants. Depending on the AMR prediction tool used. ResFinder outputs *aac(6')-aph(2'')* and AMRFinderPlus outputs *aac(6')-Ie/aph(2'')-Ia*, both perfect hits, at the same location. Alternative names: *aac(6')-aph(2'')*, *aac(6')*-bifunctional, *aac(6')-Ie*, *aacA-aphD*, *aac(6')-Ie-aph(2'')-Ia*.

[22] The annotation of gene variants varies depending on the AMR prediction tool used. For the same genomic region, ResFinder identifies the gene as *aadD*, predicting resistance to amikacin and tobramycin, which are aminoglycosides out of the scope of the DTU Genomic PT 2023. In contrast, AMRFinderPlus identifies the same gene as *aadD1* and predicts resistance to kanamycin and tobramycin. Resistance to kanamycin is known to be mediated by the enzyme Aminoglycoside O-nucleotidyltransferase ANT(4')-Ia, as reported in the literature (Pérez-Vázquez et al., 2009; Schmitz et al., 1999). However, due to the discrepancies in predicted phenotypes between the tools, participants are not penalized for omitting gene *aadD* or *aadD1* in their submission.

Table 24 – Expected results for *S. aureus* - GENOMIC23-005 (BACT/DNA).

Class	Antimi-crobial	Pred. R/S	AMR Gene or mutation	Protein name	Protein Accession no	%Identity, %Coverage	Location
Amphenicols	CHL	S	None	N/A	N/A	N/A	N/A
Beta-lactams	FOX	**R**	*mecA*	PBP2a family beta-lactam-resistant peptidoglycan transpeptidase MecA	WP_000721310.1	100, 100	Chromosome
	PEN	**R**	*mecA*	PBP2a family beta-lactam-resistant peptidoglycan transpeptidase MecA	WP_000721310.1	100, 100	Chromosome
			blaZ	BlaZ family penicillin-hydrolyzing class A beta-lactamase PC1	WP_000733283.1	100, 100	Chromosome
Folate pathway antagonists	SMX	S	None	N/A	N/A	N/A	N/A
	TMP	**R**	*dfrB* p.L21V, *dfrB* p.N60I, *dfrB* p.F99Y	Dihydrofolate reductase	WP_000175752.1	96.9, 100	Chromosome
Glycopeptides	VAN	S	None	N/A	N/A	N/A	N/A
Lincosamides	CLI	**R**	*erm(A)*	23S rRNA (adenine(2058)-N(6))-methyltransferase Erm(A)	WP_001072197.1	100, 100	Two copies on the chromosome
Macrolides	ERY	**R**	*erm(A)*	23S rRNA (adenine(2058)-N(6))-methyltransferase Erm(A)	WP_001072197.1	100, 100	Two copies on the chromosome
Oxazolidinones	LZD	S	None	N/A	N/A	N/A	N/A
Pleuromutilins	TIA	S	None	N/A	N/A	N/A	N/A
Pseudomonic acids	MUP	S	None	N/A	N/A	N/A	N/A
Quinolones	CIP	**R**	*gyrA* p.S84L	DNA gyrase subunit A	WP_000819084.1	98.9, 100	Chromosome
			parC[23] p.S80F	DNA topoisomerase IV subunit A	WP_001289586.1	99.4, 100	Chromosome

[23] In *Staphylococcus aureus*, the *parC* gene coding for the A subunit of topoisomerase IV is also known as *grlA*. Both gene names are accepted.

Table 24 – Expected results for *S. aureus* - GENOMIC23-005 (BACT/DNA).

Class	Antimi-crobial	Pred. R/S	AMR Gene or mutation	Protein name	Protein Accession no	%Identity, %Coverage	Location
Rifamycins	RIF	S	None	N/A	N/A	N/A	N/A
Steroids	FUS	S	None	N/A	N/A	N/A	N/A
Tetracyclines	TET	**R**	*tet(M)*	Tetracycline resistance ribosomal protection protein Tet(M)	WP_000691737.1	100, 100	Chromosome
			tet(38) (optional) [24]	Tetracycline efflux MFS transporter Tet(38)	WP_001100289.1	100, 100	Chromosome

[24] The Tet(38) tetracycline efflux MFS transporter is not included in the ResFinder database and, therefore, is not detected by the tool. However, it is identified by AMRFinderPlus. Consequently, in the current PT, the omission of the *tet(38)* gene is not penalized.

7.2. Evaluation of submitted results

7.2.1. AMR mutations

Table 25 – Submitted AMR mutations for *S. aureus* - GENOMIC23-005.

Class	Gene	Mutation	No of labs		SCORE		Comment
			BACT	DNA	If submitted	If not submitted	
Beta-lactams	*pbp2*	A285P	1	1	0	N/A	Not expected
		A576S	1	1	0	N/A	Not expected
		A606D	1	1	0	N/A	Not expected
		E315A	1	1	0	N/A	Not expected
	pbp4	L234H	1	1	0	N/A	Out of scope[25]
Folate pathway antagonists	*dfrB*	F99Y	24	27	**1**	0	Expected
		L21V	21	23	**1**	0	Expected
		N60I	21	23	**1**	0	Expected
		I97T	1	1	0	N/A	Out of scope[25]
		V72E	1	1	0	N/A	Out of scope[25]
Pseudomonic acids	*ileS*	I712L	1	1	0	N/A	Out of scope[25]
		N213D	1	1	0	N/A	Out of scope[25]
Quinolones	*grlA*	S80F	22	24	**1**	0	Expected
		S84L	2	1	0	N/A	Not expected
		E638K	1	1	0	N/A	Out of scope[25]
		V694M	1	1	0	N/A	Out of scope[25]
	grlB	D530G	1	1	0	N/A	Out of scope[25]
	gyrA	S84L	22	26	**1**	0	Expected
		D402E	1	1	0	N/A	Out of scope[25]
		D816E	1	1	0	N/A	Not expected
		E815D	1	1	0	N/A	Not expected
		E859V	1	1	0	N/A	Out of scope[25]
		S80F	1	2	0	N/A	Not expected
		T818del	1	1	0	N/A	Not expected
		T825_S826del	1	1	0	N/A	Not expected
		V598I	1	1	0	N/A	Out of scope[25]
	parC	S80F	2	3	**1**	0	Expected

[25] The reported mutation is present in the genome, but it is not known to be related to AMR; therefore, it is out of the scope of the DTU Genomic proficiency test and should not have been reported.

7.2.2. AMR Genes

Table 26 – Submitted AMR genes for *S. aureus* - GENOMIC23-005.

Class	Gene	No of labs		SCORE		
		BACT	DNA	If submitted	If not submitted	Comment
Aminoglycosides	*aac(6')-aph(2'')*	22	25	**1**	0	Expected
	ant(9)-Ia	9	9	0	N/A	Out of scope[26]
	aadD	8	8	**1**	**Blank**	Accepted[22]
	bleO	5	5	0	N/A	Out of scope[26]
	aph(2'')-Ia	2	2	0	N/A	Not expected
	aac(6')-Ie	2	2	**1**	0	Expected
	aadD1	2	3	**1**	**Blank**	Accepted[22]
	aac(6')-Ie-aph(2'')-Ia	1	1	**1**	0	Expected
	aacA-aphD	0	1	**1**	0	Expected
Beta-lactams	*mecA*	26	29	**1**	0	Expected
	blaZ	23	26	**1**	0	Expected
	blaPC1[27]	2	2	**1**	0	Expected
	$bla_{\text{RAHN-1}}$	1	0	0	N/A	Not expected
	mecA1	1	0	0	N/A	Not expected
	blaL	0	1	0	N/A	Not expected
Macrolides, Lincosamides, Streptogramin B	*erm(A)*	25	27	**1**	0	Expected
Tetracyclines	*tet(M)*	25	27	**1**	0	Expected
	tet(38)	3	4	**1**	**Blank**	Accepted[24]
	tet(S/M)	1	1	0	N/A	Not expected

[26] The AMR gene is present in the genome, but it is related to an AMR phenotype which is out of the scope of the DTU Genomic PT 2023, *i.e.*, it is not in the panel antimicrobials, as presented in **Table 2**. Therefore, it should not have been reported.
[27] *blaPC1* is an alternative nomenclature for *blaZ*, which is expected.

7.2.3. Predicted AMR phenotype

Table 27 – Submitted predicted AMR phenotype for *S. aureus* - GENOMIC23-005.

Class	Predicted R phenotype to antimicrobial	No of labs		SCORE		Comment
		BACT	DNA	If submitted	If not submitted	
Aminoglycosides	Gentamicin	24	27	**1**	0	Expected
	Kanamycin	17	19	**1**	0	Expected
	Streptomycin	17	17	**1**	0	Expected
Beta-lactams	Penicillin	25	28	**1**	0	Expected
	Cefoxitin	24	27	**1**	0	Expected
Folate pathway antagonists	Trimethoprim	23	26	**1**	0	Expected
	Sulfamethoxazole	2	2	0	N/A	Not expected
Lincosamides	Clindamycin	23	26	**1**	0	Expected
Macrolides	Erythromycin	24	27	**1**	0	Expected
Quinolones	Ciprofloxacin	23	26	**1**	0	Expected
Tetracyclines	Tetracycline	25	28	**1**	0	Expected

8. *S. aureus* GENOMIC23-006 (BACT/DNA)

8.1. Expected mutations, genes and predicted phenotype, related to AMR

Table 28 – Expected results for *S. aureus* - GENOMIC23-006 (BACT/DNA).

Class	Antimi-crobial	Pred. R/S	AMR Gene or mutation	Protein name	Protein Accession no.	%Identity, %Coverage	Location
Aminoglycoside	GEN	S	None	N/A	N/A	N/A	N/A
	KAN	S	None	N/A	N/A	N/A	N/A
	STR	S	None	N/A	N/A	N/A	N/A
Amphenicol	CHL	S	None	N/A	N/A	N/A	N/A
Beta-lactam	FOX	**R**	*mecA*	PBP2a family beta-lactam-resistant peptidoglycan transpeptidase MecA	WP_000721310.1	100, 100	Chromosome
	PEN	**R**	*blaZ*	Penicillin-hydrolyzing class A beta-lactamase BlaZ	WP_000733289.1	99.6, 100	Plasmid 1
			mecA	PBP2a family beta-lactam-resistant peptidoglycan transpeptidase MecA	WP_000721310.1	100, 100	Chromosome
Folate pathway antagonist	SMX	S	None	N/A	N/A	N/A	N/A
	TMP	S	None	N/A	N/A	N/A	N/A
Glycopeptide	VAN	S	None	N/A	N/A	N/A	N/A
Lincosamide	CLI	S	None	N/A	N/A	N/A	N/A
Macrolide	ERY	S	None	N/A	N/A	N/A	N/A
Oxazolidinone	LZD	S	None	N/A	N/A	N/A	N/A

Table 28 – Expected results for *S. aureus* - GENOMIC23-006 (BACT/DNA).

Class	Antimi-crobial	Pred. R/S	AMR Gene or mutation	Protein name	Protein Accession no.	%Identity, %Coverage	Location
Pleuromutilin	TIA	S	None	N/A	N/A	N/A	N/A
Pseudomonic acid	MUP	S	None	N/A	N/A	N/A	N/A
Quinolone	CIP	S	None	N/A	N/A	N/A	N/A
Rifamycin	RIF	S	None	N/A	N/A	N/A	N/A
Steroid	FUS	S	None	N/A	N/A	N/A	N/A
Tetracycline	TET	**R**	*tet(K)*	Tetracycline efflux MFS transporter Tet(K)	WP_000492283.1	100, 100	Plasmid 1
			tet(38)[28] (optional)	Tetracycline efflux MFS transporter Tet(38)	WP_001100300.1	100, 100	Chromosome

[28] The Tet(38) tetracycline efflux MFS transporter is not included in the ResFinder database and, therefore, is not detected by the tool. However, it is identified by AMRFinderPlus. Consequently, in the current PT, the omission of the *tet(38)* gene is not penalized.

8.2. Evaluation of submitted results

8.2.1. AMR mutations

Table 29 – Submitted AMR mutations for *S. aureus* - GENOMIC23-006.

Class	Gene	Mutation	No of labs		SCORE		Comment
			BACT	DNA	If submitted	If not submitted	
Beta-lactams	*pbp2*	A285P	1	1	0	N/A	Not expected
		A576S	1	1	0	N/A	Not expected
		A606D	1	1	0	N/A	Not expected
	pbp4	E398A	1	1	0	N/A	Out of scope[29]
		L234H	1	1	0	N/A	Out of scope[29]
		P253T	1	1	0	N/A	Out of scope[29]
		T25A	1	1	0	N/A	Out of scope[29]
		T409A	1	1	0	N/A	Out of scope[29]
		V202E	1	1	0	N/A	Out of scope[29]
Folate pathway antagonists	*dfrB*	F123L	1	1	0	N/A	Out of scope[29]
		I97T	1	1	0	N/A	Out of scope[29]
		V72E	1	1	0	N/A	Out of scope[29]
Pseudomonic acids	*ileS*	N213D	1	1	0	N/A	Out of scope[29]
Quinolones	*grlA*	F521Y	1	1	0	N/A	Out of scope[29]
		S309C	1	1	0	N/A	Out of scope[29]
		V694M	1	1	0	N/A	Out of scope[29]
	grlB	D530G	1	1	0	N/A	Out of scope[29]
	gyrA	A814T	1	1	0	N/A	Out of scope[29]
		D483E	1	1	0	N/A	Out of scope[29]

8.2.2. AMR Genes

Table 30 – Submitted AMR genes for *S. aureus* - GENOMIC23-006.

Class	Gene	No of labs		SCORE		Comment
		BACT	DNA	If submitted	If not submitted	
Aminoglycosides	*aph(3'')-Ib*	1	0	0	N/A	Not expected
	aph(6)-Id	1	0	0	N/A	Not expected
	aac(3)-IId	1	0	0	N/A	Not expected
	aadA5	1	0	0	N/A	Not expected
Beta-lactams	*mecA*	26	29	**1**	0	Expected
	blaZ	25	28	**1**	0	Expected
	$bla_{CMY-145}$	1	0	0	N/A	Not expected

[29] The reported mutation is present in the genome, but it is related to an AMR phenotype which is out of the scope of the DTU Genomic PT 2023, *i.e.*, it is not in the panel antimicrobials, as presented in **Table 2**. Therefore, it should not have been reported.

Table 30 – Submitted AMR genes for *S. aureus* - GENOMIC23-006.

Class	Gene	No of labs		SCORE		Comment
		BACT	DNA	If submitted	If not submitted	
	bla$_{CTX-M-15}$	1	0	0	N/A	Not expected
	blaL	1	1	0	N/A	Not expected
	bla$_{TEM-1}$	1	0	0	N/A	Not expected
Macrolides	*mph(A)*	1	0	0	N/A	Not expected
Tetracyclines	*tet(K)*	25	28	**1**	0	Expected
	tet(38)	2	4	**1**	**Blank**	Accepted[28]
	tet(B)	1	0	0	N/A	Not expected

8.2.3. Predicted AMR phenotype

Table 31 – Submitted predicted AMR phenotype for *S. aureus* - GENOMIC23-006.

Class	Predicted R phenotype to antimicrobial	No of labs		SCORE		Comment
		BACT	DNA	If submitted	If not submitted	
Aminoglycosides	Gentamicin	1	0	0	N/A	Not expected
	Streptomycin	1	0	0	N/A	Not expected
Beta-lactams	Cefoxitin	25	27	**1**	0	Expected
	Penicillin	25	28	**1**	0	Expected
Folate pathway antagonists	Trimethoprim	1	0	0	N/A	Not expected
Macrolides	Erythromycin	1	0	0	N/A	Not expected
Tetracyclines	Tetracycline	26	28	**1**	0	Expected

9. INCOMPATIBILITY (INC) GROUPS

9.1. Expected data

Expected incompatibility groups for each strain of the DTU Genomic PT 2023 are presented in **Table 32**. Differences in results when using PlasmidFinder and MOB-typer (part of the MOB-suite) can arise from variations in their databases and detection algorithms, which affect their sensitivity and specificity. Additionally, the cut-off values for identity and coverage, whether selected by the user or preset by the tool, and the different assembly and polishing tools can also influence the final output. Unlike antibiotics, where a phenotype can often be directly linked to a genotype, such a direct correlation is not straightforward with incompatibility groups. As a result, one tool might identify certain replicons that another does not, or classify them differently, leading to differences in the analysis.

The expected data were based on both PlasmidFinder and MOB-Recon outputs from the closed genomes. Results with higher than 95% identity and 90% coverage were included in the expected data. However, since participants employed short-read WGS data for the plasmid incompatibility groups analysis, results with lower coverage were also accepted, since the region used to characterize them could be split and consequently span different contigs.

Table 32 – Plasmid incompatibility (Inc) groups identified in the reference genomes of the DTU Genomic PT strains, including their genomic location and the corresponding percentage identity and coverage obtained using PlasmidFinder analysis.

Organism	Strain	Incompatibility group (Inc)	Location	% Identity	% Coverage
E. coli	GENOMIC23-001	IncFIB(AP001918)	Plasmid 2	97.68	100[30]
		IncHI2	Plasmid 1	100	100
		IncHI2A	Plasmid 1	99.52	100
		IncX1	Plasmid 3	98.93	100
		IncX4	Plasmid 4	100	100
	GENOMIC23-002	Col156	Plasmid 5	97.4	100
		IncFIB(AP001918)	Plasmid 2	97.68	100[31]
		IncFII(pCoo)	Plasmid 3	97.68	100[31]
		IncI1-I(Alpha)	Plasmid 1	100	100
		IncX1	Plasmid 4	98.93	100
S. enterica	GENOMIC23-003	IncFIB(AP001918)	Plasmid 2	97.7	100[30]
		IncHI2	Plasmid 1	100	100
		IncHI2A	Plasmid 1	100	100
	GENOMIC23-004	Col440II	Plasmid 3	100	100
		ColpVC	Plasmid 6	97.4	100
		IncC	Plasmid 1	100	100
		IncM2	Plasmid 2	100	100[30]
S. aureus	GENOMIC23-005	rep22_repB(pAMalpha1)	Chromosome	99.8	100
	GENOMIC23-006	rep20_rep(pTW20)	Plasmid 1	100	100[30]
		rep5a_ repSAP001(pN315)	Plasmid 1	100	100

[30] 100% coverage was obtained before the reorientation of the plasmid with Dnaapler.

9.2. Evaluation of submitted data

Table 33 – Submitted replicons for each strain.

Organism	Strain	Replicon type	No of labs		SCORE		Comment
			BACT	DNA	If reported	If not reported	
E. coli	GENOMIC23-001	IncFIB(AP001918)	22	25	**1**	0	Expected
		IncHI2	25	30	**1**	0	Expected
		IncHI2A	23	27	**1**	0	Expected
		IncX1	24	29	**1**	0	Expected
		IncX4	26	30	**1**	0	Expected
		Col(MG828)	8	11	**1**	**Blank**	Accepted[31]
		IncFIA	1	1	**1**	**Blank**	Accepted[32]
		IncFIC(FII)	7	7	**1**	**Blank**	Accepted
		Col156	2	0	0	N/A	Not expected
		ColpVC	0	1	0	N/A	Not expected
		IncFII(pCoo)	1	0	0	N/A	Not expected
		IncL	1	0	0	N/A	Not expected
		IncX3	1	1	0	N/A	Not expected[33]
	GENOMIC23-002	Col156	22	24	**1**	0	Expected
		IncFIB(AP001918)	22	24	**1**	0	Expected
		IncFII(pCoo)	21	24	**1**	0	Expected
		IncI1-I(Alpha)	23	26	**1**	0	Expected
		IncX1	20	27	**1**	0	Expected
		ColKP3	17	21	**1**	**Blank**	Accepted
		ColRNAI	3	3	**1**	**Blank**	Accepted
		IncFIA	1	1	**1**	**Blank**	Accepted
		IncFIC(FII)	5	5	**1**	**Blank**	Accepted
		IncI(Gamma)	1	1	**1**	**Blank**	Accepted
		ColpVC	0	1	0	N/A	Not expected
		IncFII	1	0	0	N/A	Not expected
S. enterica	GENOMIC23-003	IncFIB(AP001918)	21	22	**1**	0	Expected
		IncHI2	23	23	**1**	0	Expected
		IncHI2A	25	27	**1**	0	Expected
		IncFIA	1	1	**1**	**Blank**	Accepted[32]
		IncFIC(FII)	7	7	**1**	**Blank**	Accepted[34]
		IncQ1	17	16	**1**	**Blank**	Accepted[34]
	GENOMIC23-004	Col440II	22	24	**1**	0	Expected
		ColpVC	20	19	**1**	0	Expected
		IncC	24	25	**1**	0	Expected
		IncM2	23	24	**1**	0	Expected

[31] Outputted by PlasmidFinder, but not MOB-suite.

[32] Outputted by MOB-suite, but not PlasmidFinder.

[33] Hit with 82.5% identity and 228/374 (61%) coverage.

[34] Hits with percent coverage below the applied threshold of 95% identity or 90% coverage. Accepted as participants use short-read (Illumina) data for the analysis.

		Col(pHAD28)	4	5	**1**	N/A	Accepted[35]
		ColRNAI	3	3	**1**	N/A	Accepted[36]
		IncA	1	1	0	N/A	Not expected
		IncFIB(AP001918)	1	1	0	N/A	Not expected
		IncFIC(FII)	1	0	0	N/A	Not expected
		IncHI2	1	1	0	N/A	Not expected
		IncHI2A	1	1	0	N/A	Not expected
		IncL	2	2	0	N/A	Not expected
		IncQ1	1	1	0	N/A	Not expected
S. aureus	GENOMIC23-005	rep22_repB(pAMalpha1)	18	20	**1**	0	Expected
		rep7a_ORF(pKH1)	3	3	0	N/A	Not expected
		rep2(pYSI8)	1	1	0	N/A	Not expected
		repI(pGB354)	1	1	0	N/A	Not expected
	GENOMIC23-006	rep20_rep(pTW20)	18	20	**1**	0	Expected
		rep5a_ repSAP001(pN315)	16	17	**1**	0	Expected
		rep7a_repC(pS0385p1)	11	11	**1**	**Blank**	Accepted[37]
		rep7a_ repC(Cassette)	8	9	**1**	**Blank**	Accepted[37]
		rep(pN315)	3	4	0	N/A	Not expected
		rep(pS0385)	2	2	0	N/A	Not expected
		rep(pACK4)	0	1	0	N/A	Not expected
		rep2(pYSI8)	0	1	0	N/A	Not expected

10. SUPPLEMENTARY DATA – AMR PHENOTYPE

Determination of the Minimum Inhibitory Concentration (MIC) of the *E. coli* and *Salmonella* test strains for each antimicrobial was performed using the Sensititre panels EUVSEC3 and EUVSEC2, while for *S. aureus* using EUST2 (Trek Diagnostic Systems Ltd, UK). The expected MIC values were generated by performing Broth Microdilution (BMD) for all test strains in two different occasions at the Technical University of Denmark, National Food Institute (DTU Food). The method applied for the BMD was the ISO 20776-1 “Susceptibility testing of infectious agents and evaluation of performance of antimicrobial susceptibility test devices”.

MIC results were interpreted based on Epidemiological Cut-off (ECOFF) values established by the European Committee on Antimicrobial Susceptibility Testing (EUCAST, www.eucast.org) and strains were categorised as Wild-type or Non-Wild-type for each antimicrobial.

[35] Hit for Col(pHAD28) with 92.4% identity and 100% coverage.

[36] Hit for ColRNAI with 88.6% identity and 101.5% coverage.

[37] Rep7a outputted by PlasmidFinder with identity of 100%, and coverage of 89.5% (repC(pS0385p1)) and 71.0% (repC(Cassette)). Participants might have not reported it due to applying more strict thresholds in their pipeline. Therefore, participants who reported rep7a were scored with a “1”; however, scores were blanked for participants who did not report this plasmid replicon.

10.1. *E. coli* GENOMIC23-001

Class	Antimicrobial	MIC	ECOFF	Wild-type (WT)/Non-WT
Aminoglycosides	Amikacin	≤4	8	WT
	Gentamicin	>16	2	**Non-WT**
Amphenicols	Chloramphenicol	=32	16	**Non-WT**
Beta-lactams	Ampicillin	>32	8	**Non-WT**
	Cefepime	=4	0.125	**Non-WT**
	Cefotaxime	>4	0.25	**Non-WT**
	Cefoxitin	=4	16	WT
	Ceftazidime	=4	1	**Non-WT**
	Ertapenem	≤0.015	0.03	WT
	Imipenem	≤0.125	0.5	WT
	Meropenem	≤0.03	0.06	WT
	Temocillin	=8	16	WT
Folate pathway antagonists	Sulfamethoxazole	>512	64	**Non-WT**
	Trimethoprim	>16	2	**Non-WT**
Macrolides	Azithromycin	=8	16	WT
Polymyxins	Colistin	=8	2	**Non-WT**
Quinolones	Ciprofloxacin	>8	0.06	**Non-WT**
	Nalidixic acid	>64	8	**Non-WT**
Tetracyclines	Tetracycline	>32	8	**Non-WT**
	Tigecycline	≤0.25	0.5	WT

10.2. *E. coli* GENOMIC23-002

Class	Antimicrobial	MIC	ECOFF	Wild-type (WT)/Non-WT
Aminoglycosides	Amikacin	≤4	8	WT
	Gentamicin	>16	2	**Non-WT**
Amphenicols	Chloramphenicol	>64	16	**Non-WT**
Beta-lactams	Ampicillin	>32	8	**Non-WT**
	Cefepime	=1	0.125	**Non-WT**
	Cefotaxime	>4	0.25	**Non-WT**
	Cefoxitin	>64	16	**Non-WT**
	Ceftazidime	>8	1	**Non-WT**
	Ertapenem	=2	0.03	**Non-WT**
	Imipenem	=0.5	0.5	WT
	Meropenem	=0.5	0.06	**Non-WT**
	Temocillin	>128	16	**Non-WT**
Folate pathway antagonists	Sulfamethoxazole	>512	64	**Non-WT**
	Trimethoprim	>16	2	**Non-WT**
Macrolides	Azithromycin	=8	16	WT
Polymyxins	Colistin	≤1	2	WT
Quinolones	Ciprofloxacin	>8	0.06	**Non-WT**
	Nalidixic acid	>64	8	**Non-WT**
Tetracyclines	Tetracycline	>32	8	**Non-WT**
	Tigecycline	≤0.25	0.5	WT

10.3. *S. enterica* GENOMIC23-003

Class	Antimicrobial	MIC	ECOFF	Wild-type (WT)/Non-WT
Aminoglycosides	Amikacin	≤4	4	WT
	Gentamicin	>16	2	**Non-WT**
Amphenicols	Chloramphenicol	>64	16	**Non-WT**
Beta-lactams	Ampicillin	>32	4	**Non-WT**
	Cefepime	=1	0.25	**Non-WT**
	Cefotaxime	=4	0.5	**Non-WT**
	Cefoxitin	=2	8	WT
	Ceftazidime	>8	2	**Non-WT**
	Ertapenem	≤0.015	0.03125	WT
	Imipenem	=0.25	1	WT
	Meropenem	≤0.03	0.125	WT
	Temocillin	=8	16	WT
Folate pathway antagonists	Sulfamethoxazole	>512	256	**Non-WT**
	Trimethoprim	>16	2	**Non-WT**
Macrolides	Azithromycin	=8	16	WT
Polymyxins	Colistin	≤1	2	WT
Quinolones	Ciprofloxacin	=0.03	0.125	WT
	Nalidixic acid	=8	8	WT
Tetracyclines	Tetracycline	=4	8	WT
	Tigecycline	=0.5	0.5	WT

10.4. *S. enterica* GENOMIC23-004

Class	Antimicrobial	MIC	ECOFF	Wild-type (WT)/Non-WT
Aminoglycosides	Amikacin	>128	4	**Non-WT**
	Gentamicin	>16	2	**Non-WT**
Amphenicols	Chloramphenicol	=64	16	**Non-WT**
Beta-lactams	Ampicillin	>32	4	**Non-WT**
	Cefepime	>32	0.25	**Non-WT**
	Cefotaxime	>4	0.5	**Non-WT**
	Cefoxitin	>64	8	**Non-WT**
	Ceftazidime	>8	2	**Non-WT**
	Ertapenem	>2	0.03125	**Non-WT**
	Imipenem	=4	1	**Non-WT**
	Meropenem	=16	0.125	**Non-WT**
	Temocillin	>128	16	**Non-WT**
Folate pathway antagonists	Sulfamethoxazole	=512	256	**Non-WT**
	Trimethoprim	≤0.25	2	WT
Macrolides	Azithromycin	>64	16	**Non-WT**
Polymyxins	Colistin	≤1	2	WT
Quinolones	Ciprofloxacin	>8	0.125	**Non-WT**
	Nalidixic acid	>64	8	**Non-WT**
Tetracyclines	Tetracycline	=4	8	WT
	Tigecycline	≤0.25	0.5	WT

10.5. *S. aureus* GENOMIC23-005

Class	Antimicrobial	MIC	ECOFF	Wild-type (WT)/Non-WT
Aminoglycosides	Gentamicin	=8	2	**Non-WT**
	Kanamycin	>32	8	**Non-WT**
	Streptomycin	>32	16	**Non-WT**
Amphenicols	Chloramphenicol	=8	16	WT
Beta-lactams	Cefoxitin	>16	4	**Non-WT**
	Penicillin	>1	0.125	**Non-WT**
Folate pathway antagonists	Sulfamethoxazole	=512	128	**Non-WT**
	Trimethoprim	>16	2	**Non-WT**
Glycopeptides	Vancomycin	≤1	2	WT
Lincosamides	Clindamycin	>4	0.25	**Non-WT**
Macrolides	Erythromycin	>8	1	**Non-WT**
Oxazolidinones	Linezolid	≤1	4	WT
Pleuromutilins	Tiamulin	≤0.5	2	WT
Pseudomonic acids	Mupirocin	≤0.5	1	WT
Quinolones	Ciprofloxacin	>8	2	**Non-WT**
Rifamycins	Rifampin	≤0.015	0.03125	WT
Steroid antibacterials	Fusidate	≤0.25	0.5	WT
Tetracyclines	Tetracycline	>16	1	**Non-WT**

10.6. *S. aureus* GENOMIC23-006

Class	Antimicrobial	MIC	ECOFF	Wild-type (WT)/Non-WT
Aminoglycosides	Gentamicin	≤0.5	2	WT
	Kanamycin	≤4	8	WT
	Streptomycin	=8	16	WT
Amphenicols	Chloramphenicol	=8	16	WT
Beta-lactams	Cefoxitin	>16	4	**Non-WT**
	Penicillin	>1	0.125	**Non-WT**
Folate pathway antagonists	Sulfamethoxazole	≤64	128	WT
	Trimethoprim	≤1	2	WT
Glycopeptides	Vancomycin	≤1	2	WT
Lincosamides	Clindamycin	=0.25	0.25	WT
Macrolides	Erythromycin	=0.5	1	WT
Oxazolidinones	Linezolid	=2	4	WT
Pleuromutilins	Tiamulin	=1	2	WT
Pseudomonic acids	Mupirocin	≤0.5	1	WT
Quinolones	Ciprofloxacin	=0.5	2	WT
Rifamycins	Rifampin	≤0.015	0.03125	WT
Steroid antibacterials	Fusidate	=0.5	0.5	WT
Tetracyclines	Tetracycline	>16	1	**Non-WT**

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