DTU Genomic PT 2021

A guide on interpretation of submitted data and scoring system

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1. Foreword

This document is provided to the EURL-AR and SEQAFRICA networks, participants of the DTU Genomic proficiency test (PT) 2021, as a guide to assist in the performance evaluation of the antimicrobial resistance (AMR) part of the PT. In the following sections, a short introduction and overviews of the submitted data (anonymized) are presented for each AMR category, i.e., chromosomal mutations, genes and gene variants, as well as the predicted AMR phenotype. Moreover, the scoring system and overviews of the obtained scores are presented in detail. Please note that participants' evaluation is not expressed on a pass/fail basis, and the participants are therefore encouraged to self-evaluate their performance applying internal acceptance criteria. Finally, the present document offers a preliminary data analysis, as a draft manuscript is in preparation.

2. Introduction

The participants of Genomic PT 2021 were requested to perform whole genome sequencing and bioinformatics analysis on two strains of each of the following species: *Salmonella enterica* (n=2), *Escherichia coli* (n=2) and *Campylobacter coli/C. jejuni* (n=2). The two *Campylobacter* strains employed in the Genomic PT 2021 belonged to the species *C. jejuni* and will be referred to as *C. jejuni* for the rest of the document. The codes used for each strains in Genomic PT 2021 are presented in Table 1. For each strain, two types of DNA samples were analysed: 1) DNA isolated from live cultures, referred to as "BACT" and 2) pre-prepared DNA, referred to as "DNA". The downstream processing of the samples is presented in Figure 1.

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

Table 1. Strains included in Genomic PT 2021. Two types of samples were analysed for each strain: DNA isolated from live cultures (BACT) and pre-prepared DNA (DNA).

Species	Strain code	Material
S. enterica	GENOMIC21-001	BACT/DNA
	GENOMIC21-002	BACT/DNA
E. coli	GENOMIC21-003	BACT/DNA
	GENOMIC21-004	BACT/DNA
C. jejuni	GENOMIC21-005	BACT/DNA
	GENOMIC21-006	BACT/DNA

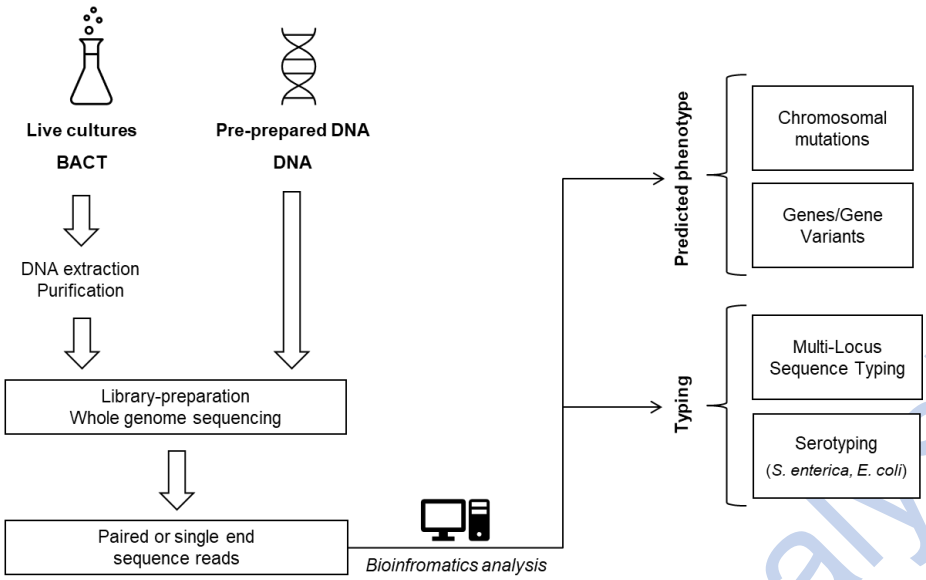


Figure 1. Schematic overview of the downstream processing of live culture samples (BACT) and pre-prepared DNA samples (DNA).

Table 2. Overview of antimicrobials included in Genomic PT 2021, for S. enterica, E. coli and C. jejuni.

Antimicrobial	Abbreviation	Class	*S. enterica*	*E. coli*	*C. jejuni*
Amikacin	AMI	Aminoglycoside	X	X	
Ampicillin	AMP	β-Lactam	X	X	
Azithromycin	AZI	Macrolide	X	X	
Cefepime	FEP	β-Lactam	X	X	
Cefotaxime	FOT	β-Lactam	X	X	
Cefoxitin	FOX	β-Lactam	X	X	
Ceftazidime	TAZ	β-Lactam	X	X	
Chloramphenicol	CHL	Amphenicol	X	X	X
Ciprofloxacin	CIP	Quinolone	X	X	X
Colistin	COL	Polymyxin	X	X	
Ertapenem	ETP	β-Lactam	X	X	X
Erythromycin	ERY	Macrolide	X	X	X
Gentamicin	GEN	Aminoglycoside	X	X	X
Imipenem	IMI	β-Lactam	X	X	
Kanamycin	KAN	Aminoglycoside	X	X	
Meropenem	MERO	β-Lactam	X	X	
Nalidixic acid	NAL	Quinolone	X	X	
Rifampicin	RIF	Rifamycin	X	X	
Streptomycin	STR	Aminoglycoside	X	X	
Sulfamethoxazole	SMX	Folate pathway antagonist	X	X	
Tetracycline	TET	Tetracycline	X	X	X
Tigecycline	TGC	Tetracycline	X	X	
Trimethoprim	TMP	Folate pathway antagonist	X	X	

3. Participation in DTU Genomic PT 2021

There are 21 participants in the EURL-AR and SEQAFRICA networks, which were assigned codes starting from 2021-13 to 2021-33. The level of participation of the 21 laboratories in the analysis of the different materials included in Genomic PT 2021 (n_{total}=12) is presented in Table 3 and Figure 2. Two laboratories (2021-25 and 2021-32) registered, but did not participate in Genomic PT 2021. Laboratories 2021-13, 2021-15, 2021-28, 2021-30 and 2021-33 analysed 50-83% of the samples (Figure 2). The rest of the participants signed up for all materials provided in the PT.

Table 3. Overview of participation in the different materials included in Genomic PT 2021.Grey=not participated.

	S. enterica				*E. coli*				*C. jejuni*			
	GENOMIC21-001		GENOMIC21-002		GENOMIC21-003		GENOMIC21-004		GENOMIC21-005		GENOMIC21-006	
	BACT	DNA	BACT	DNA	BACT	DNA	BACT	DNA	BACT	DNA	BACT	DNA
2021-13	x		x		x		x		x		x	
2021-14	x	x	x	x	x	x	x	x	x	x	x	x
2021-15	x	x	x	x					x	x	x	x
2021-16	x	x	x	x	x	x	x	x	x	x	x	x
2021-17	x	x	x	x	x	x	x	x	x	x	x	x
2021-18	x	x	x	x	x	x	x	x	x	x	x	x
2021-19	x	x	x	x	x	x	x	x	x	x	x	x
2021-20	x	x	x	x	x	x	x	x	x	x	x	x
2021-21	x	x	x	x	x	x	x	x	x	x	x	x
2021-22	x	x	x	x	x	x	x	x	x	x	x	x
2021-23	x	x	x	x	x	x	x	x	x	x	x	x
2021-24	x	x	x	x	x	x	x	x	x	x	x	x
2021-25												
2021-26	x	x	x	x	x	x	x	x	x	x	x	x
2021-27	x	x	x	x	x	x	x	x	x	x	x	x
2021-28	x	x	x	x	x	x	x	x				
2021-29	x	x	x	x	x	x	x	x	x	x	x	x
2021-30	x	x	x	x	x	x	x					
2021-31	x	x	x	x	x	x	x	x	x	x	x	x
2021-32												
2021-33	x	x	x	x	x	x	x	x		x		x

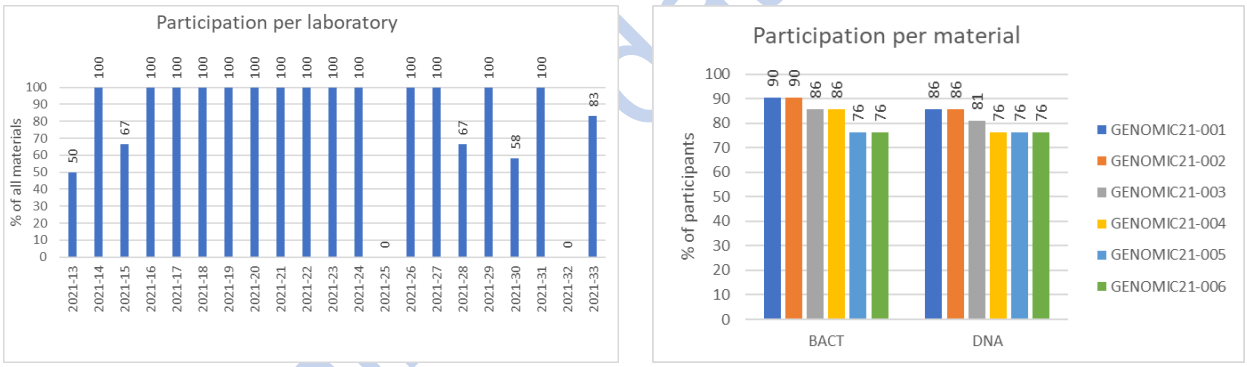


Figure 2. Percent of materials (n_{total}=12) analysed by each laboratory in Genomic PT 2021 (Left) and percent of participants that analysed each sample (Right).

4. Analysis of AMR determinants and predicted AMR phenotype

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

The expected results for each strain, related to the identification of AMR determinants, as well as the predicted phenotypic AMR profile, are summarized in Table 4. The expected results were re-evaluated after data submission by the participants and the outcome of this re-evaluation was to exclude some results, i.e., blank

the relevant scores. An overview of the excluded results, for which the scores are blanked, is presented in Table 5 and in the list below:

1. In the reference genome, gene *tet(M)* in *S. enterica* strain GENOMIC21-001 is present with 96% identity, which was evaluated as too low; therefore, *tet(M)* was excluded from the list of expected genes. Since there is not a universal cutoff value for the lowest accepted percent identity, participants who reported the *tet(M)* gene were not scored with zero.
2. All AMR results for *S. enterica* strain GENOMIC21-002 were not evaluated. The majority of participants did not identify three AMR genes present on plasmid (*blaCTX-M-1*, *dfrA1* and *dfrA14*) in DNA isolated from cultures (BACT sample). A possible explanation is that the plasmid was lost during culturing and therefore it is not meaningful to evaluate these results. Moreover, we were not able to identify some of the expected resistance genes in the pre-prepared DNA that was sent to the participants; therefore, these results were also excluded.
3. In Appendix 2 of Genomic PT 2021 protocol[1], on page 7, it is stated that "All genes conferring resistance to the above-mentioned classes of antimicrobials should be reported". The intention was to report all genes conferring resistance to the antimicrobials mentioned in Table 1 of Appendix 2 of the protocol (footnote 1, page 6), and not the respective classes in general. Some participants submitted resistance genes for the two *C. jejuni* strains GENOMIC21-005 and GENOMIC21-006, which are related to resistance to β-Lactams, but not ETP, which is the only β-Lactam included in this PT for *C. jejuni*. For this reason, it was decided to exclude these results from evaluation; therefore, the respective scores were blanked.
4. Results regarding the chromosomal mutation T86I in *C. jejuni* strain GENOMIC21-006 are not evaluated because of issues regarding the reference *gyrA* gene used in the ResFinder database. Predicted phenotype resistant to CIP is not evaluated too, i.e., phenotypes resistant or susceptible to CIP were accepted.
5. In the reference genome, gene *tet(O)* in *C. jejuni* strain GENOMIC21-006 is present with 95% identity, which was evaluated as too low; therefore, scores for reporting the *tet(O)* gene were blanked and predicted phenotypes resistant or susceptible to TET were accepted.

[1] PROTOCOL for DTU Genomic Proficiency Test 2021, 2021, EURL-AR.

Table 4. Expected AMR results for each strain. The AMR determinants marked in yellow, and the respective AMR phenotypes are excluded from evaluation (scores are blanked) – see text. Gene aac(6')-Iaa is a cryptic gene in Salmonella; therefore, even though it is present in the sequences of both Salmonella strains included in this PT, it is not expected to confer resistance to AMI phenotypically.

Strain code	AMR Determinant	Type	Antimicrobial	Antimicrobial Class	Predicted phenotype R/S
GENOMIC21-001	*aph(3')-Ia*	Gene/Gene Var.	KAN	Aminoglycoside	**R**
	blaTEM-1A/B/C/D	Gene/Gene Var.	AMP	β-Lactam	**R**
	cmlA1	Gene/Gene Var.	CHL	Amphenicol	**R**
	dfrA12	Gene/Gene Var.	TMP	Folate pathway antagonist	**R**
	mcr-1.1	Gene/Gene Var.	COL	Polymyxin	**R**
	mef(B)	Gene/Gene Var.	AZI ERY	Macrolide	**R**
	sul3	Gene/Gene Var.	SMX	Folate pathway antagonist	**R**
	tet(B)	Gene/Gene Var.	TET	Tetracycline	**R**
	aac(6')-Iaa	Gene/Gene Var.	AMI	Aminoglycoside	**S**
	aadA1, ant(3'')-Ia	Gene/Gene Var.	STR	Aminoglycoside	**R**
	aadA2, aadA2b	Gene/Gene Var.	STR	Aminoglycoside	**R**
GENOMIC21-003	*blaNDM-4*	Gene/Gene Var.	AMP, FEP, FOT, FOX, TAZ, ETP, IMI, MERO	β-Lactam	**R**
	blaTEM-1B	Gene/Gene Var.	AMP	β-Lactam	**R**
	dfrA12	Gene/Gene Var.	TMP	Folate pathway antagonist	**R**
	sul1	Gene/Gene Var.	SMX	Folate pathway antagonist	**R**
	sul3	Gene/Gene Var.	SMX	Folate pathway antagonist	**R**
	aadA2, adA2b	Gene/Gene Var.	STR	Aminoglycoside	**R**
GENOMIC21-004	*aph(3'')-Ib*	Gene/Gene Var.	STR	Aminoglycoside	**R**
	aph(6)-Id	Gene/Gene Var.	STR	Aminoglycoside	**R**
	sul2	Gene/Gene Var.	SMX	Folate pathway antagonist	**R**
	tet(B)	Gene/Gene Var.	TET	TET	**R**
GENOMIC21-005	A2075G	Chr. Mutation	ERY	Macrolide, 23S rRNA	**R**
	tet(O)	Gene/Gene Var.	TET	TET	**R**
GENOMIC21-006	A2075G	Chr. Mutation	ERY	Macrolide, 23S rRNA	**R**
	T86I	Chr. Mutation	CIP	Quinolone, gyrA	**R**
	aph(2'')-If	Gene/Gene Var.	GEN	Aminoglycoside	**R**
	cat	Gene/Gene Var.	CHL	Amphenicol	**R**
	tet(O)	Gene/Gene Var.	TET	Tetracycline	**R**
	aac(6')-ph(2'')	Gene/Gene Var.	GEN	Aminoglycoside	**R**

Table 5. Overview of changes in expected results and re-evaluation of the respective scores.

Strain	AMR determinant or AMR phenotype	Change
GENOMIC21-001	Gene *tet(M)*	Scores are blanked.
GENOMIC21-002	All AMR determinants (chromosomal mutations, gene/gene variant, predicted AMR phenotype)	Scores are blanked.
GENOMIC21-005	Genes *blaOXA-61*, *blaOXA-193*, *blaOXA-450*, *blaOXA-451*, *blaOXA-452*, *blaOXA-453*, *blaOXA-489*	Scores are blanked.
GENOMIC21-006	Chromosomal mutation T86I	Scores are blanked.
	Genes *ant(6)-Ia*, *aph(3')-III*, *blaOXA-61*, *blaOXA-193*, *blaOXA-450*, *blaOXA-451*, *blaOXA-452*, *blaOXA-453*, *blaOXA-489, tet(O)*	Scores are blanked.
	Predicted phenotype R to CIP	Phenotypes with or without CIP accepted (score=1)
	Predicted phenotype R to TET	Phenotypes with or without TET accepted (score=1)

S. enterica, GENOMIC21-001

From the 21 laboratories, 19 analysed GENOMIC21-001-BACT and 18 GENOMIC21-001-DNA. An overview of the submitted data about AMR genes or gene variants for *S. enterica* strain GENOMIC21-001 is presented in Table 6 and Figure 3. Six of the expected genes, *blaTEM-1A/B/C/D*, *cmlA1*, *dfrA12*, *mef(B)*, *sul3* and *tet(B)*) were identified by all participants that signed up for this strain in both BACT and DNA. The rest of the expected genes were identified by 74-84% of the participants. Genes *aph(3')-Ia*, *aadA1* and *aadA2* are on a plasmid, which could have been lost during culturing; however, the fact that these genes were not identified in the DNA sample either, suggests against this argument.

Almost 80% of the participants reported gene *tet(M)*, related to phenotypic resistance to TET, which was not in the list of expected genes. This gene was identified in the reference genome too; however, the percent identity value to the reference gene in the ResFinder database (%ID=96) was considered too low to be reliable, and it was therefore excluded from the list of expected genes. Since a universal cutoff value for the lowest reliable percent identity has not been identified yet, scores regarding the *tet(M)* gene in strain GENOMIC21-001 were blanked, i.e., participants did not get a zero score for reporting the *tet(M)* gene. Other deviating genes (*aph(3'')-Ia*, *mcr-1.26*, *aac(6')-Ia* and *aac(6')-IIa*) were reported by a small fraction of participants (5-6%) but were not identified by the EURL-AR pipeline in the reference genome; therefore, reporting of these genes was scored with zero (score=0). Reporting of the deviating gene *aph(3'')-Ia* by 2021-28 is likely a typo, as the expected gene *aph(3')-Ia* is identified in the submitted sequences of this participant by the EURL-AR pipeline. Reporting of *aac(6')-Ia* (laboratory 2021-33) and *aac(6')-IIa* (laboratory 2021-29) is likely a typo too, because in the submitted sequences from these participants the expected gene *aac(6')-Iaa* is identified by the EURL-AR pipeline. Moreover the sequences of genes *aac(6')-Ia* and *aac(6')-IIa* are very different to the expected gene *aac(6')-Iaa*. Gene *mcr-1.26*, reported by laboratory 2021-30, is in the ResFinder database, and is likely one amino acid shorted than the wildtype gene. There is a mutation in the first codon, which could interfere with translation; however, the second codon in the sequence is an ATG too. Reporting of *mcr-1.26* was evaluated as a mistake because it disregards parts of the gene sequence; however, this can be a limitation of the bioinformatics tool used, depending on how the tool chooses the best matching reference. It could also be a consequence of other sequencing parameters (DNA extraction, sequencer, etc.).

Table 6. Gene/gene variant data submitted by each participant for the live culture (BACT) and pre-prepared DNA (DNA) of S. enterica strain GENOMIC21-001. Grey: deviating results, crossed cells: laboratory did not participate.

	BACT																					DNA																	
	2021-13	2021-14	2021-15	2021-16	2021-17	2021-18	2021-19	2021-20	2021-21	2021-22	2021-23	2021-24	2021-25	2021-26	2021-27	2021-28	2021-29	2021-30	2021-31	2021-32	2021-33	2021-13	2021-14	2021-15	2021-16	2021-17	2021-18	2021-19	2021-20	2021-21	2021-22	2021-23	2021-24	2021-25	2021-26	2021-27	2021-28	2021-29	2021-30	2021-31	2021-32	2021-33
aph(3')-Ia	x		x	x	x	x	x	x		x	x	x		x	x		x	x			x			x	x	x	x	x	x		x	x	x		x	x	x	x	x			x
blaTEM1A/B/C/D	x	x	x	x	x	x	x	x	x	x	x	x		x	x	x	x	x	x		x		x	x	x	x	x	x	x	x	x	x		x	x	x	x	x	x		x
cmlA1	x	x	x	x	x	x	x	x	x	x	x	x		x	x	x	x	x	x		x		x	x	x	x	x	x	x	x	x	x		x	x	x	x	x	x		x
dfrA12	x	x	x	x	x	x	x	x	x	x	x	x		x	x	x	x	x	x		x		x	x	x	x	x	x	x	x	x	x		x	x	x	x	x	x		x
mcr-1.1	x	x	x	x	x	x	x	x		x	x	x		x	x	x	x		x		x		x	x	x	x	x	x	x		x	x	x		x	x	x	x		x		x
mef(B)	x	x	x	x	x	x	x	x	x	x	x	x		x	x	x	x	x	x		x		x	x	x	x	x	x	x	x	x	x	x		x	x	x	x	x	x		x
sul3	x	x	x	x	x	x	x	x	x	x	x	x		x	x	x	x	x	x		x		x	x	x	x	x	x	x	x	x	x	x		x	x	x	x	x	x		x
tet(B)	x	x	x	x	x	x	x	x	x	x	x	x		x	x	x	x	x	x		x		x	x	x	x	x	x	x	x	x	x	x		x	x	x	x	x	x		x
aac(6')-Iaa	x	x	x	x	x	x	x	x		x	x	x			x	x			x				x	x	x	x	x	x	x		x	x	x			x	x	x	x			
aadA1, ant(3'')-Ia	x		x	x	x	x	x	x		x	x	x		x	x	x	x	x			x			x	x	x	x	x	x		x	x	x			x	x	x	x			x
aadA2, aadA2b	x		x	x	x	x	x	x		x	x	x		x	x	x	x	x			x			x	x	x	x	x	x		x	x	x			x	x	x	x			x
aph(3'')-Ia																x																											
mcr-1.26																		x																					x				
tet(M)	x			x	x	x	x	x	x	x		x			x	x	x	x	x		x				x	x	x	x	x	x	x		x			x	x	x	x	x		x
aac(6')-Ia																					x																						x
aac(6')-IIa																	x																										

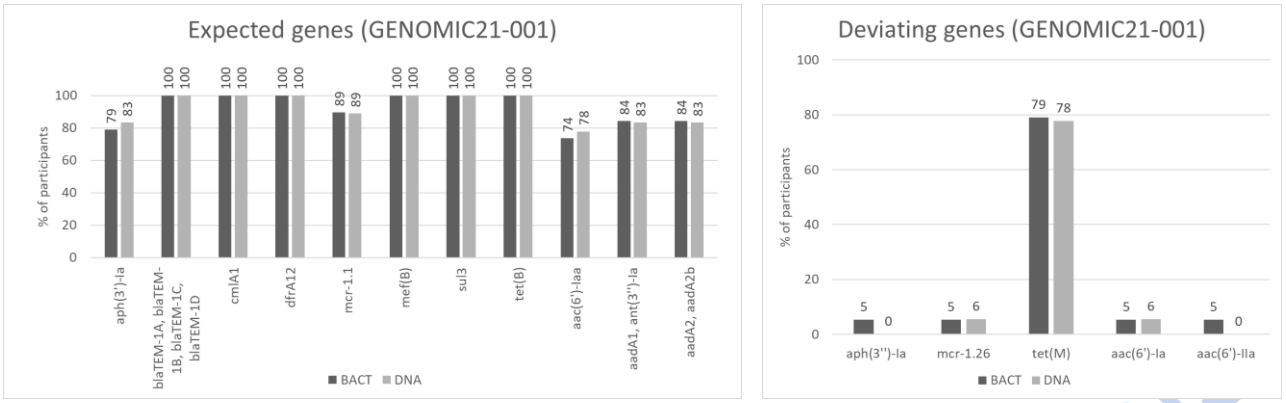


Figure 3. Percent of participants reporting expected (Left) and deviating (Right) genes for BACT and DNA samples for S. enterica strain GENOMIC21-001.

An overview of the submitted data about the predicted AMR phenotype for *S. enterica* strain GENOMIC21-001 is presented in Table 7 and Figure 4. Eight participants reported the complete expected AMR phenotypic profile, for both BACT and DNA, plus resistance to AMI. Two participants (2021-21 and 2021-29) did not submit any data, either for BACT or for DNA material. The rest of the participants did not identify one, or more of the expected AMR phenotypes. Even though six of the expected genes (*blaTEM-1A/B/C/D*, *cmlA1*, *dfrA12*, *mef(B)*, *sul3* and *tet(B)*) were identified by all participants, the respective AMR phenotypes (AMP, CHL, TMP, AZI, SMX and TET) were reported by 83-89% of them. Similarly, even though gene *mef(B)* was reported by all laboratories, phenotypic resistance to ERY was reported by 50 and 53% for BACT and DNA material respectively. Phenotypic resistance to KAN and STR was reported by roughly 60% of the participants, even though the respective AMR genes *aph(3')-Ia* and aadA1 or aadA2 were reported by approximately 80% of the participants. Sixteen participants (84%) for BACT and 15 (83%) for DNA reported resistance to AMI, attributed to the presence of gene *aac(6')-Iaa*; however, this is a cryptic gene in *S. enterica*, therefore it is not expected to confer phenotypic resistance to AMI. Lab 2021-30 reported phenotypic resistance to FOX in the BACT sample but not the DNA, which was evaluated as a mistake because no genetic background for resistance to FOX was identified by EURL-AR pipeline; moreover, laboratory 2021-30 did not provide any relevant genetic background either.

Table 7. Predicted AMR phenotype data submitted by each participant for the live culture (BACT) and pre-prepared DNA (DNA) of S. enterica strain GENOMIC21-001. Grey: deviating results, crossed cells: laboratory did not participate.

	BACT																					DNA																	
	2021-13	2021-14	2021-15	2021-16	2021-17	2021-18	2021-19	2021-20	2021-21	2021-22	2021-23	2021-24	2021-25	2021-26	2021-27	2021-28	2021-29	2021-30	2021-31	2021-32	2021-33	2021-13	2021-14	2021-15	2021-16	2021-17	2021-18	2021-19	2021-20	2021-21	2021-22	2021-23	2021-24	2021-25	2021-26	2021-27	2021-28	2021-29	2021-30	2021-31	2021-32	2021-33
AMP	X	X	X	X	X	X	X	X		X	X	X		X	X	X		X	X		X		X	X	X	X	X	X	X		X	X	X		X	X	X		X	X		X
AZI	X	X	X	X	X	X	X			X	X	X		X	X	X		X	X		X		X	X	X	X	X	X			X	X	X		X	X	X		X	X		X
CHL	X	X	X	X	X	X	X	X		X	X	X		X	X			X	X		X		X	X	X	X	X	X	X		X	X	X		X	X	X		X	X		X
COL	X	X	X	X	X	X	X	X		X	X	X		X	X	X		X	X		X		X	X	X	X	X	X	X		X	X	X		X	X	X		X	X		X
ERY	X		X	X	X	X	X				X			X		X					X			X	X	X	X	X				X			X		X					X
KAN			X	X	X	X	X	X		X	X			X		X					X			X	X	X	X	X	X		X	X			X		X					X
STR	X		X	X	X	X	X	X		X	X			X		X								X	X	X	X	X	X		X	X			X		X					
SMX	X	X	X	X	X	X	X	X		X	X	X		X	X	X		X	X		X			X	X	X	X	X	X		X	X	X		X	X	X		X	X		X
TET	X	X	X	X	X	X	X	X		X	X	X		X	X	X		X	X		X		X	X	X	X	X	X	X		X	X	X		X	X	X		X	X		X
TMP	X	X	X	X	X	X	X	X		X	X	X		X	X	X		X	X		X		X	X	X	X	X	X	X		X	X	X		X	X	X		X	X		X
AMI	X		X	X	X	X	X	X		X	X	X		X	X	X		X	X		X			X	X	X	X	X	X		X	X	X		X	X	X		X	X		X
FOX																		X																								

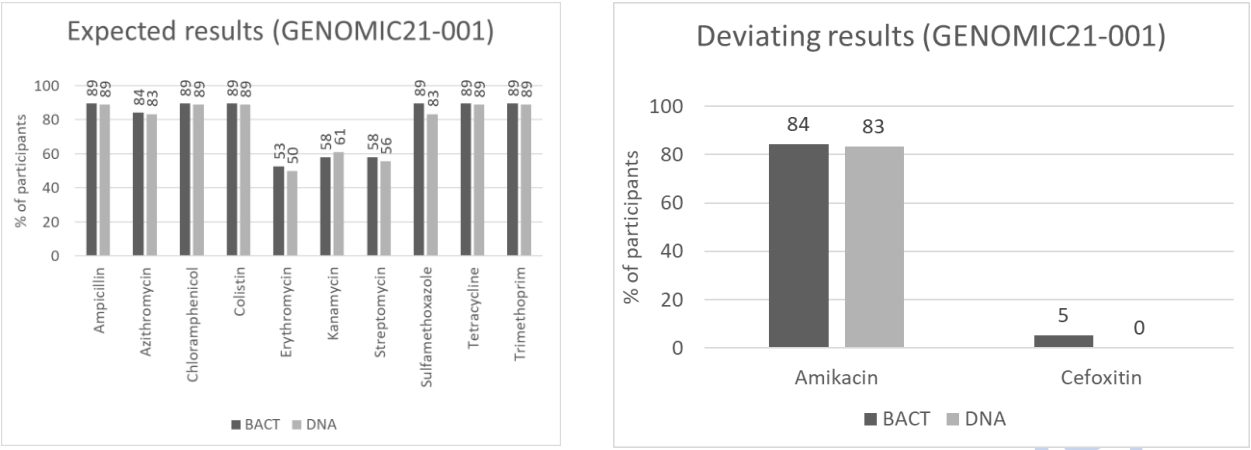


Figure 4. Percent of participants reporting expected (Left) and deviating (Right) AMR phenotypes for BACT and DNA samples for S. enterica strain GENOMIC21-001.

E. coli, GENOMIC21-003

From the 21 laboratories, 18 analysed GENOMIC21-003-BACT and 17 GENOMIC21-003-DNA. An overview of the submitted data about AMR genes or gene variants for *E. coli* strain GENOMIC21-003 is presented in Table 8 and Figure 5. Regarding the expected AMR genes, *blaNDM-4* and *sul3* were identified by all participants for both BACT and DNA. Genes *blaTEM1A/B/C/D*, *drfA12* and *sul1* were identified by 94%, while gene *aadA2* by roughly 80% of participants. Each of the deviating genes *cmr* and *mdt(A)* were reported by one participant in BACT and DNA. Gene *cmr* is an alternative name for *mdf(A)*, coding for an efflux pump known to have broad-spectrum activity. The DTU Genomic PT 2021 protocol states that *mdf(A)* should not be reported (Appendix 2, page 7 in DTU Genomic PT 2021 protocol). Gene *mdt(A)* codes for an efflux pump too, but it was not identified in the reference genome by EURL-AR pipeline. Perhaps reporting of *mdt(A)* is a typo and laboratory 2021-33 wished to report *mdf(A)* instead. Submitting *cmr* or *mdt(A)* was evaluated as a mistake.

Table 8. Gene/gene variant data submitted by each participant for the live culture (BACT) and pre-prepared DNA (DNA) of E. coli strain GENOMIC21-003. Grey: deviating results, crossed cells: laboratory did not participate.

	BACT																				DNA																
	2021-13	2021-14	2021-15	2021-16	2021-17	2021-18	2021-19	2021-20	2021-21	2021-22	2021-23	2021-24	2021-25	2021-26	2021-27	2021-28	2021-29	2021-30	2021-31	2021-32	2021-33	2021-13	2021-14	2021-15	2021-16	2021-17	2021-18	2021-19	2021-20	2021-21	2021-22	2021-23	2021-24	2021-25	2021-26	2021-27	2021-28	2021-29	2021-30	2021-31	2021-32	2021-33
blaNDM-4	x	x		x	x	x	x	x	x	x	x	x		x	x	x	x	x	x		x		x		x	x	x	x	x	x	x	x		x	x	x	x	x	x		x
blaTEM-1A/B/C/D	x	x		x	x	x		x	x	x	x	x		x	x	x	x	x	x		x		x		x	x	x		x	x	x	x	x		x	x	x	x	x		x
dfrA12	x	x		x	x	x	x	x		x	x	x		x	x	x	x	x	x		x		x		x	x	x	x	x		x	x	x		x	x	x	x	x		x
sul1	x	x		x	x	x		x	x	x	x	x		x	x	x	x	x	x		x		x		x	x	x		x	x	x	x	x		x	x	x	x	x		x
sul3	x	x		x	x	x	x	x	x	x	x	x		x	x	x	x	x	x		x		x		x	x	x	x	x	x	x	x	x		x	x	x	x	x		x
aadA2, aadA2b	x			x	x	x	x	x		x	x	x		x	x	x	x	x			x				x	x	x	x	x		x	x	x		x	x	x	x	x		x
cmr																	x																					x					
mdt(A)																					x																						x

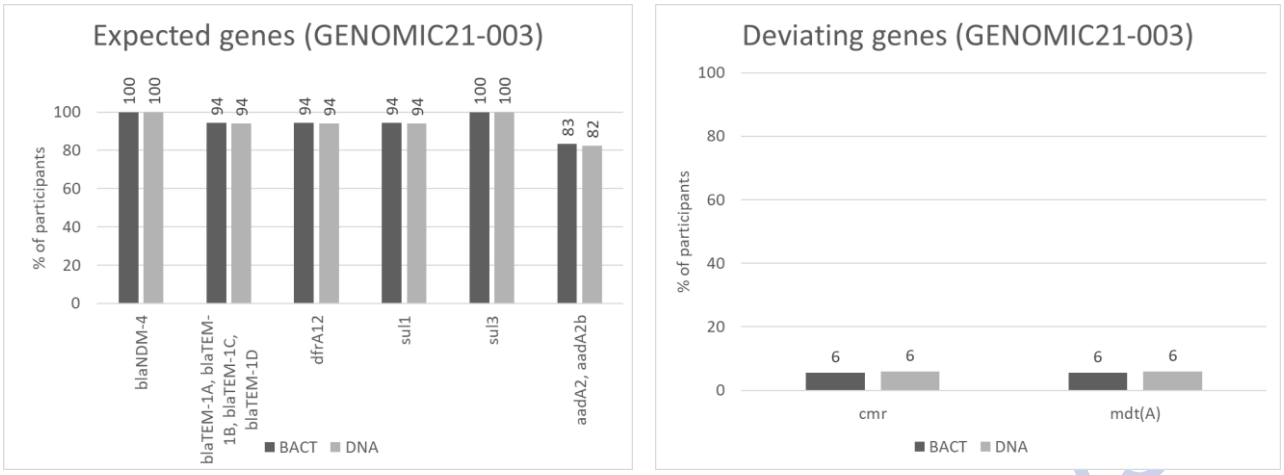


Figure 5. Percent of participants reporting expected (Left) and deviating (Right) genes for BACT and DNA samples, E. coli strain GENOMIC21-003.

An overview of the submitted data about the predicted AMR phenotype for strain GENOMIC21-003 is presented in Table 9 and Figure 6. Even though several participants reported the expected AMR genes, reporting of the respective AMR phenotype took place at a lower rate. The fact that two laboratories did not submit any data (2021-21 and 2021-29) contributed to the lower rates, but it is not the only factor observed. Gene *blaNDM-4* was reported by all participants; however, the respective AMR phenotypes (AMP, FEP, FOT, FOX, TAZ, ETP, IMI, MERO) were reported by 76-89%. Genes *sul1* and *sul3* were identified by laboratory 2021-33; however, phenotypic resistance to SMX was not reported by this laboratory, leading to a ~80% reporting of phenotypic resistance to SMX. Gene *dfrA12* was identified by 94% of the participants; however, the respective phenotypic resistance to TMP was reported by 83-88%. Phenotypic resistance to STR was reported by ~50% of participants even though the respective AMR gene *aadA2* was identified by ~80% of the participants. Seven participants submitted the entire expected AMR phenotypic profile for both BACT and DNA material. One participant reported resistance to GEN (2021-30), and one to TET (2021-13). Both cases were not supported by a relevant genetic background. In addition, AMR determinants conferring phenotypic resistance to these antimicrobials were not identified in the reference genome by the EURL-AR pipeline; therefore, reporting phenotypic resistance to GEN or TET was evaluated as a mistake (score=0).

Table 9. Predicted AMR phenotype data submitted by each participant for the live culture (BACT) and pre-prepared DNA (DNA) of E. coli strain GENOMIC21-003. Grey: deviating results, crossed cells: laboratory did not participate.

	BACT																				DNA																
	2021-13	2021-14	2021-15	2021-16	2021-17	2021-18	2021-19	2021-20	2021-21	2021-22	2021-23	2021-24	2021-25	2021-26	2021-27	2021-28	2021-29	2021-30	2021-31	2021-32	2021-33	2021-13	2021-14	2021-15	2021-16	2021-17	2021-18	2021-19	2021-20	2021-21	2021-22	2021-23	2021-24	2021-25	2021-26	2021-27	2021-28	2021-29	2021-30	2021-31	2021-32	2021-33
AMP	X	X		X	X	X	X	X		X	X	X		X	X	X			X		X		X		X	X	X	X	X		X	X	X		X	X	X		X	X		X
FEP	X	X		X	X	X	X	X		X	X	X		X	X	X		X	X		X		X		X	X	X	X	X		X	X	X		X	X	X		X	X		X
FOT	X	X		X	X		X	X		X	X	X		X	X	X		X	X		X		X		X	X		X	X		X	X	X		X	X	X			X		X
FOX	X	X		X	X	X	X	X		X	X	X		X	X	X		X	X		X		X		X	X	X	X	X		X	X	X		X	X	X		X	X		X
TAZ	X	X		X	X	X	X	X		X	X	X		X	X	X		X	X		X		X		X	X	X	X	X		X	X	X		X	X	X		X	X		X
ETP	X	X		X	X	X	X	X		X	X	X		X	X	X		X	X		X		X		X	X	X	X	X		X	X	X		X	X	X		X	X		X
IMI	X	X		X	X	X	X	X		X	X	X		X	X	X		X	X		X		X		X	X	X	X	X		X	X	X		X	X	X		X	X		X
MERO	X	X		X	X	X	X	X		X	X	X		X	X	X		X	X		X		X		X	X	X	X	X		X	X	X		X	X	X		X	X		X
STR	X			X	X	X	X	X		X	X			X											X	X	X	X	X		X	X			X							
SMX	X	X		X	X	X	X	X		X	X	X		X	X	X		X	X				X		X	X	X	X	X		X	X	X		X	X	X		X	X		
TMP		X		X	X	X	X	X		X	X	X		X	X	X		X	X		X		X		X	X	X	X	X		X	X	X		X	X	X		X	X		X
GEN																																						X			
TET	X																																									

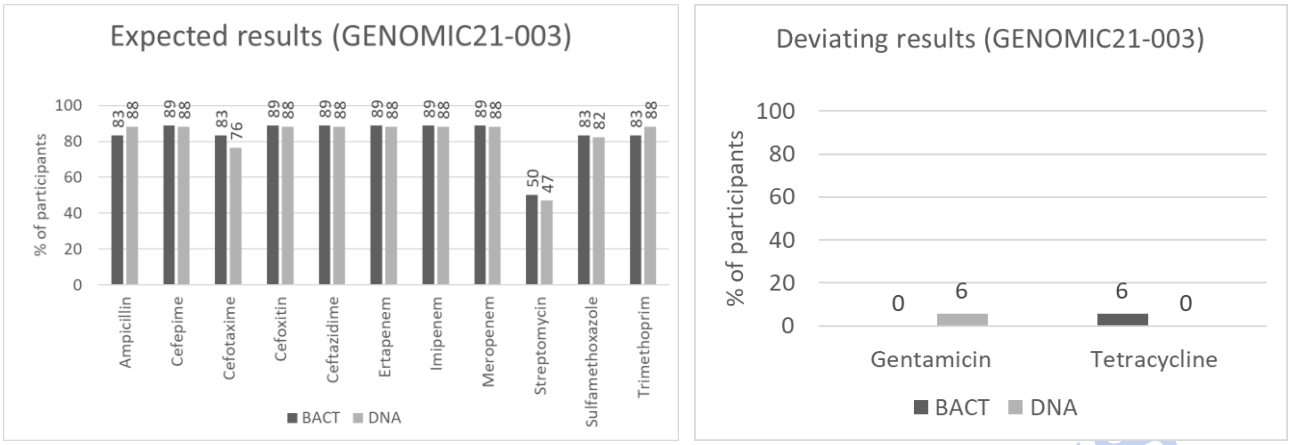


Figure 6. Percent of participants reporting expected (Left) deviating (Right) AMR phenotypes for BACT and DNA samples for E. coli strain GENOMIC21-003.

E. coli, GENOMIC21-004

From the 21 laboratories, 18 analysed GENOMIC21-004-BACT and 16 GENOMIC21-004-DNA. An overview of the submitted data about strain GENOMIC21-004 is presented in Table 10 and Figure 7 (AMR genes) and in Table 11 and Figure 8 (predicted AMR phenotype). Regarding the expected genes, gene *sul2* and *tet(B)* were identified by all participants in BACT and by 94% in DNA; however, the respective phenotypic resistance to SMX or TET were reported by 81-89% of the participants. Genes *aph(3'')-Ib* and *aph(6)-Id* were identified by 69-83% of participants and the respective phenotypic resistance to STR was reported by 56-67%. Each of the following three deviating genes, *aph(6)-Ib*, *cmr* and *mdt(A)*, were reported by one participant and were evaluated as mistakes. Reporting of *aph(6)-Ib* could be due to typo, as the name is similar to the expected gene *aph(6)-Id*, and *aph(6)-Ib* was not identified in the reference genome by the EURL-AR pipeline. Similarly to strain GENOMIC21-003, reporting of *cmr* and *mdt(A)* was evaluated as a mistake – see explanation above for strain GENOMIC21-003.

Table 10. Gene/gene variant data submitted by each participant for the live culture (BACT) and pre-prepared DNA (DNA) of E. coli strain GENOMIC21-004. Grey: deviating results, crossed cells: laboratory did not participate.

	BACT																					DNA																
	2021-13	2021-14	2021-15	2021-16	2021-17	2021-18	2021-19	2021-20	2021-21	2021-22	2021-23	2021-24	2021-25	2021-26	2021-27	2021-28	2021-29	2021-30	2021-31	2021-32	2021-33	2021-13	2021-14	2021-15	2021-16	2021-17	2021-18	2021-19	2021-20	2021-21	2021-22	2021-23	2021-24	2021-25	2021-26	2021-27	2021-28	2021-29	2021-30	2021-31	2021-32	2021-33
aph(3'')-Ib	x			x	x	x	x	x		x	x	x		x	x	x	x	x			x				x	x	x	x	x			x	x		x	x	x	x			x
aph(6)-Id	x			x	x	x		x		x	x	x		x	x	x	x	x			x				x	x	x		x			x	x		x	x	x	x			x
sul2	x	x		x	x	x	x	x	x	x	x	x		x	x	x	x	x	x		x		x		x	x	x	x	x			x	x		x	x	x	x	x		x
tet(B)	x	x		x	x	x	x	x	x	x	x	x		x	x	x	x	x	x		x		x		x	x	x	x	x			x	x		x	x	x	x		x	x
aph(6)-Ib							x																					x													
cmr																	x																						x			
mdt(A)																					x																					x

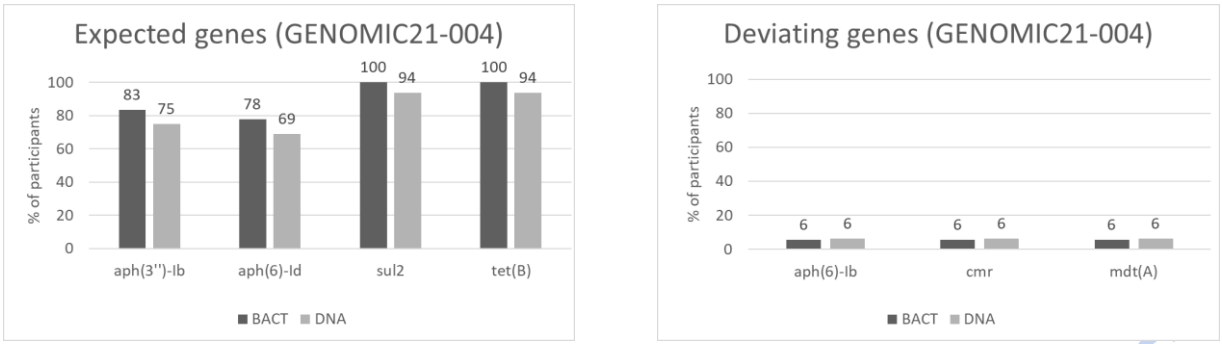


Figure 7. Percent of participants reporting expected (Left) and deviating (Right) AMR genes for BACT and DNA samples, E. coli strain GENOMIC21-004.

Table 11. Predicted phenotype data submitted by each participant for the live culture (BACT) and pre-prepared DNA (DNA) of E. coli strain GENOMIC21-004. Grey: deviating results, crossed cells: laboratory did not participate.

	BACT																				DNA															
	2021-13	2021-14	2021-15	2021-16	2021-17	2021-18	2021-19	2021-20	2021-21	2021-22	2021-23	2021-24	2021-25	2021-26	2021-27	2021-28	2021-29	2021-30	2021-31	2021-32	2021-33	2021-13	2021-14	2021-15	2021-16	2021-17	2021-18	2021-19	2021-20	2021-21	2021-22	2021-23	2021-24	2021-25	2021-26	2021-27	2021-28	2021-29	2021-30	2021-31	2021-32	2021-33
STR	x		/	x	x	x	x	x		x	x		/	x		x		x		/	x	/		/	x	x	x	x	x			x		/	x		x		/		/	x
SMX	x	x	/	x	x	x	x	x		x	x	x	/	x	x	x		x	x	/	x	/	x	/	x	x	x	x	x			x	x	/	x	x	x		/	x	/	x
TET	x	x	/	x	x	x	x	x		x	x	x	/	x	x	x			x	/	x	/	x	/	x	x	x	x	x			x	x	/	x	x	x		/	x	/	x

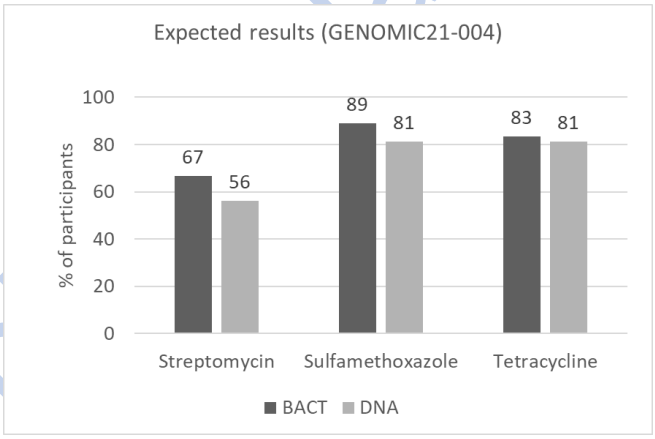


Figure 8. Percent of participants reporting expected AMR phenotypes for BACT and DNA samples for E. coli strain GENOMIC21-004. Deviating AMR phenotypes were not reported for this strain.

C. jejuni, GENOMIC21-005

From the 21 laboratories, 16 analysed both GENOMIC21-005-BACT and GENOMIC21-005-DNA. An overview of the submitted data is presented in Table 12 (chromosomal mutations), Table 13 and Figure 9 (AMR genes) and in Table 14 and Figure 10 (predicted AMR phenotype). All participants reported mutation A2075G in 23S rRNA, in both BACT and DNA; however, the respective phenotypic resistance to ERY was reported by 81% of the participants. All participants reported the expected gene *tet(O)*; however, 81% reported phenotypic resistance to TET. Several participants reported the deviating genes *blaOXA-61* and *blaOXA193* (56 and 31% respectively). Other deviating genes (*blaOXA-45*, *blaOXA-451*, *blaOXA-452*, *blaOXA-453* and *blaOXA-489*) were reported by two participants. The above mentioned blaOXA genes confer resistance to β-Lactams and are indeed present in the reference genome; however, blaOXA genes are not known to confer resistance to ETP, which was the only β-Lactam included in this proficiency test for *C. jejuni* (Table 2). Therefore, these genes

should not have been reported. However, since the Genomic PT 2021 protocol was not providing clear information on this issue, it was decided to blank the scores for these results (see also § 4 and Table 5). Phenotypic resistance to ETP and GEN was reported by one laboratory each, and were both evaluated as mistakes, because a relevant genetic background was not identified in the reference genome by the EURL-AR pipeline

Table 12. Chromosomal mutation data submitted by each participant for the live culture (BACT) and pre-prepared DNA (DNA) of C. jejuni strain GENOMIC21-005. Crossed cells: laboratory did not participate.

	BACT																				DNA																	
	2021-13	2021-14	2021-15	2021-16	2021-17	2021-18	2021-19	2021-20	2021-21	2021-22	2021-23	2021-24	2021-25	2021-26	2021-27	2021-28	2021-29	2021-30	2021-31	2021-32	2021-33	2021-13	2021-14	2021-15	2021-16	2021-17	2021-18	2021-19	2021-20	2021-21	2021-22	2021-23	2021-24	2021-25	2021-26	2021-27	2021-28	2021-29	2021-30	2021-31	2021-32	2021-33
A275G	x	x	x	x	x	x	x	x	x	x	x	x		x	x		x		x				x	x	x	x	x	x	x	x	x	x		x	x		x		x		x

Table 13. Gene/Gene variant data submitted by each participant for the live culture (BACT) and pre-prepared DNA (DNA) of C. jejuni strain GENOMIC21-005. Grey: deviating results, crossed cells: laboratory did not participate.

	BACT																					DNA																	
	2021-13	2021-14	2021-15	2021-16	2021-17	2021-18	2021-19	2021-20	2021-21	2021-22	2021-23	2021-24	2021-25	2021-26	2021-27	2021-28	2021-29	2021-30	2021-31	2021-32	2021-33	2021-13	2021-14	2021-15	2021-16	2021-17	2021-18	2021-19	2021-20	2021-21	2021-22	2021-23	2021-24	2021-25	2021-26	2021-27	2021-28	2021-29	2021-30	2021-31	2021-32	2021-33
tet(O)	x	x	x	x	x	x	x	x	x	x	x	x		x	x		x		x				x	x	x	x	x	x	x	x	x	x					x		x		x
blaOXA-193			x	x	x	x											x							x	x	x	x											x				
blaOXA-45						x											x										x											x				
blaOXA-451						x											x										x											x				
blaOXA-452						x											x										x											x				
blaOXA-453						x											x										x											x				
blaOXA-489						x											x										x											x				
blaOXA-61	x					x	x	x		x		x		x			x		x								x	x	x		x		x		x		x		x		x

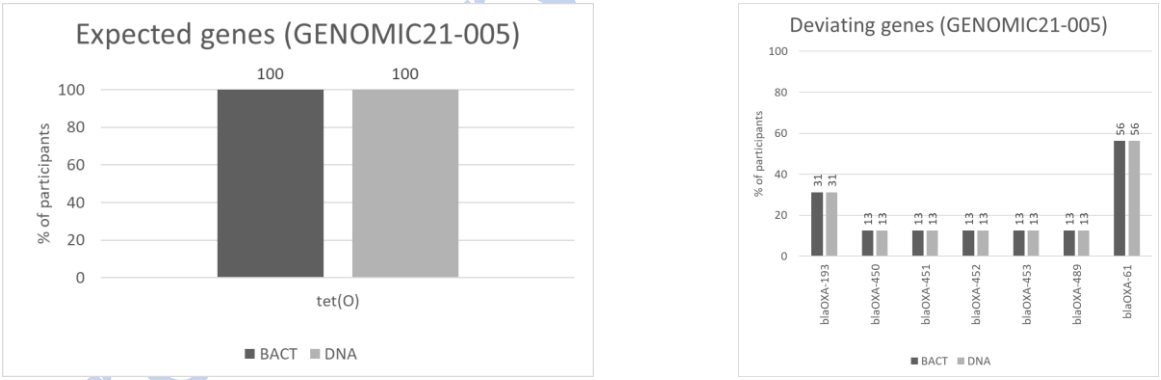


Figure 9. Percent of participants reporting expected (Left) and deviating (Right) genes for BACT and DNA samples, C. jejuni strain GENOMIC21-005.

Table 14. Predicted phenotype data submitted by each participant for the live culture (BACT) and pre-prepared DNA (DNA) of C. jejuni strain GENOMIC21-005. Grey: deviating results, crossed cells: laboratory did not participate.

	BACT																					DNA																	
	2021-13	2021-14	2021-15	2021-16	2021-17	2021-18	2021-19	2021-20	2021-21	2021-22	2021-23	2021-24	2021-25	2021-26	2021-27	2021-28	2021-29	2021-30	2021-31	2021-32	2021-33	2021-13	2021-14	2021-15	2021-16	2021-17	2021-18	2021-19	2021-20	2021-21	2021-22	2021-23	2021-24	2021-25	2021-26	2021-27	2021-28	2021-29	2021-30	2021-31	2021-32	2021-33
ERY	x	x	x	x	x	x	x	x		x	x			x	x				x				x	x	x	x	x	x	x		x	x			x	x			x		x
TET	x	x	x	x	x	x	x	x		x	x			x	x				x				x	x	x	x	x	x	x		x	x			x	x			x		x
ETP				x																																						
GEN																																										x

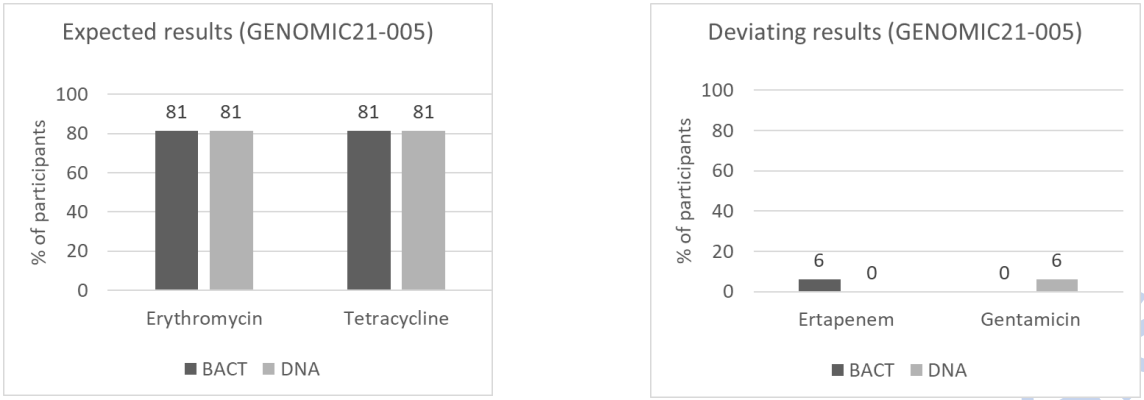


Figure 10. Percent of participants reporting expected (Left) and deviating (Right) predicted AMR phenotypes for BACT and DNA samples for C. jejuni, strain GENOMIC21-005.

C. jejuni, GENOMIC21-006

From the 21 laboratories, 16 analysed both GENOMIC21-006-BACT and GENOMIC21-006-DNA. An overview of the submitted data for strain GENOMIC21-006 is presented in Table 15 (chromosomal mutations), Table 16 and Figure 11 (AMR genes) and Table 17 and Figure 12 (predicted AMR phenotype). All participating laboratories identified mutation A2075G in 23S rRNA, in both BACT and DNA; however, 75% of the participants reported the respective predicted phenotypic resistance to ERY. Results regarding the chromosomal mutation T86I in *gyrA*, reported by 44% of the participants, were not evaluated because of issues regarding the reference *gyrA* gene used in the ResFinder database. Phenotypic resistance to CIP, reported by 38% of the participants, was not evaluated too, i.e., profiles with or without CIP were accepted (see also § 4 and Table 5). One participant reported a deviating chromosomal mutation in *gyrA* (E59L), which was evaluated as a mistake because it was not identified in the reference genome.

Table 15. Chromosomal mutation data submitted by each participant for the live culture (BACT) and pre-prepared DNA (DNA) of C. jejuni strain GENOMIC21-006. Grey: deviating results, crossed cells: laboratory did not participate.

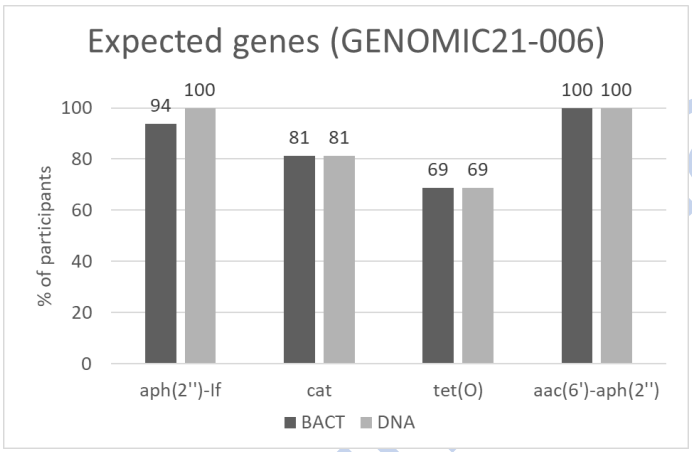
	BACT																				DNA																
	2021-13	2021-14	2021-15	2021-16	2021-17	2021-18	2021-19	2021-20	2021-21	2021-22	2021-23	2021-24	2021-25	2021-26	2021-27	2021-28	2021-29	2021-30	2021-31	2021-32	2021-33	2021-13	2021-14	2021-15	2021-16	2021-17	2021-18	2021-19	2021-20	2021-21	2021-22	2021-23	2021-24	2021-25	2021-26	2021-27	2021-28	2021-29	2021-30	2021-31	2021-32	2021-33
A2075G	X	X	X	X	X	X	X	X	X	X	X	X		X	X		X		X				X	X	X	X	X	X	X	X	X	X		X	X		X		X		X
T86I			X		X	X	X			X		X			X									X		X	X	X			X		X			X					
E59L				X																					X																

All participants identified the expected gene ***aac(6')-aph(2'')***; however, the respective phenotypic resistance to GEN was reported by 81% of the participants. Gene *aph(2'')-If*, also conferring resistance to GEN, was reported by all participants in the DNA sample, and by all but one participant in the BACT sample. Phenotypic resistance to CHL and TET was reported by 56% of the participants, while the respective AMR genes *cat* and *tet(O)* were identified by 81 and 69% of the participants, respectively. In the reference genome, *tet(O)* gene is present with 95% identity to the reference gene in ResFinder database, which was evaluated by EURL-AR, at a later stage, as too low; therefore, scores for reporting *tet(O)* gene were blanked and predicted phenotypes resistant or susceptible to TET were accepted. The majority of participants reported the deviating AMR genes, *ant(6)-Ia* (88%), *aph(3')-III* (75%), predicted to confer phenotypic resistance to aminoglycosides but neither of them is known to confer resistance to GEN, which is the only aminoglycoside included in Genomic PT 2021 for *C. jejuni* (Table 2). In addition, many participants reported one or more of blaOXA genes (*blaOXA*-61, blaOXA-*193*, *blaOXA-450*, *blaOXA-451*, *blaOXA-452*, *blaOXA-453* and *blaOXA-489*). Similarly to strain GENOMIC21-005, blaOXA genes are known to confer resistance to β-Lactams, but not ETP, which is the only β-Lactam antibiotic included in Genomic PT 2021 for *C. jejuni* (Table 2). However, since the protocol did not provide clear instructions on this, the scores for these results are blanked (see also § 4 and Table 5). Phenotypic resistance

to ETP was reported by one participant and it was evaluated as a mistake, because a relevant genetic background was not identified by the EURL-AR pipeline in the reference genome.

Table 16. Gene/gene variant data submitted by each participant for the live culture (BACT) and pre-prepared DNA (DNA) of C. jejuni strain GENOMIC21-006. Grey: deviating results, crossed cells: laboratory did not participate.

	BACT																				DNA																	
	2021-13	2021-14	2021-15	2021-16	2021-17	2021-18	2021-19	2021-20	2021-21	2021-22	2021-23	2021-24	2021-25	2021-26	2021-27	2021-28	2021-29	2021-30	2021-31	2021-32	2021-33	2021-13	2021-14	2021-15	2021-16	2021-17	2021-18	2021-19	2021-20	2021-21	2021-22	2021-23	2021-24	2021-25	2021-26	2021-27	2021-28	2021-29	2021-30	2021-31	2021-32	2021-33
aph(2'')-If	x	x	x	x	x	x	x	x	x	x	x	x		x	x				x				x	x	x	x	x	x	x	x	x	x		x	x		x		x		x
cat	x		x	x	x	x	x	x		x	x	x		x	x		x							x	x	x	x	x		x	x	x		x	x		x				x
tet(O)	x			x	x	x	x	x	x	x		x			x				x						x	x	x	x	x	x	x		x			x		x		x
aac(6')-aph(2'')	x	x	x	x	x	x	x	x	x	x	x	x		x	x		x		x				x	x	x	x	x	x	x	x	x	x	x			x		x		x
ant(6)-Ia	x	x	x	x	x	x		x	x	x	x	x		x			x		x				x	x	x	x	x		x	x	x	x	x			x		x		x
aph(3')-III	x		x	x	x	x		x		x	x	x		x	x		x							x	x	x		x		x	x	x		x			x		x		x
blaOXA-193			x	x	x	x											x							x	x	x	x														
blaOXA-450						x											x										x														
blaOXA-451						x											x										x														
blaOXA-452						x											x										x														
blaOXA-453						x											x										x														
blaOXA-489						x											x										x														
blaOXA-61	x					x	x	x	x	x		x		x			x		x							x	x	x	x	x		x		x			x		x		x



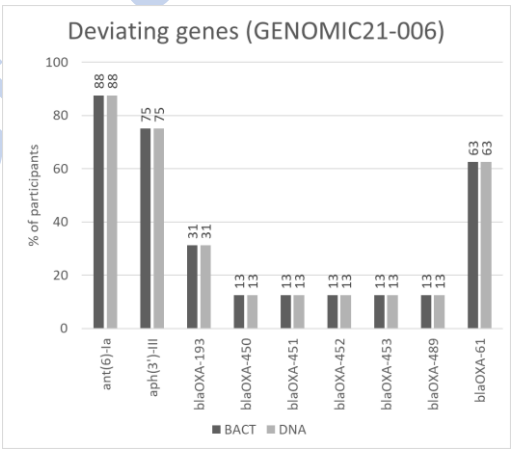


Figure 11. Percent of participants reporting expected (Left) and deviating (Right) genes for BACT and DNA samples, C. jejuni strain GENOMIC21-006.

Table 17. Predicted phenotype data submitted by each participant for the live culture (BACT) and pre-prepared DNA (DNA) of C. jejuni GENOMIC21-006. Grey: deviating results, crossed cells: laboratory did not participate.

	BACT																				DNA																	
	2021-13	2021-14	2021-15	2021-16	2021-17	2021-18	2021-19	2021-20	2021-21	2021-22	2021-23	2021-24	2021-25	2021-26	2021-27	2021-28	2021-29	2021-30	2021-31	2021-32	2021-33	2021-13	2021-14	2021-15	2021-16	2021-17	2021-18	2021-19	2021-20	2021-21	2021-22	2021-23	2021-24	2021-25	2021-26	2021-27	2021-28	2021-29	2021-30	2021-31	2021-32	2021-33
CHL	x		x	x	x		x	x		x	x			x							x			x	x	x		x	x		x	x			x							x
CIP			x	x	x		x			x					x									x	x	x		x			x					x					
ERY	x	x	x	x	x		x	x		x	x			x	x				x				x	x	x	x		x	x		x	x			x	x		x		x	
GEN	x	x	x	x	x	x	x	x		x	x			x	x				x				x	x	x	x	x	x	x		x	x			x	x		x		x	
TET	x			x	x	x	x	x		x					x				x						x	x	x	x	x		x					x			x		x
ETP				x																					x																

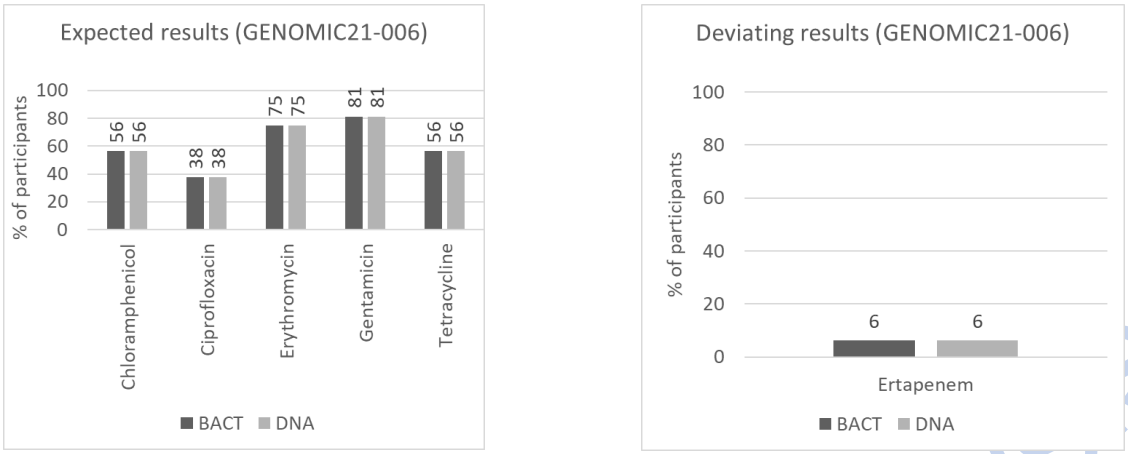


Figure 12. Percent of participants reporting expected resistant phenotypes for BACT and DNA samples for C. jejuni strain GENOMIC21-006.

5. Performance of participants on the identification of AMR determinants and predicted AMR phenotype

The performance of the participants regarding the identification of AMR determinants was expressed as percent of the maximum possible score that could be obtained for chromosomal mutations, or genes and genes variants, for each strain – see Table 18.

Performance on chromosomal mutation identification, per strain

The performance of the participants regarding the identification of chromosomal mutations is presented in Figure 13. Scores were blanked for mutation T86I in strain GENOMIC21-006 (see §4 and Table 5), i.e., data were excluded from the evaluation. All participating laboratories achieved the maximum score for identification of chromosomal mutations.

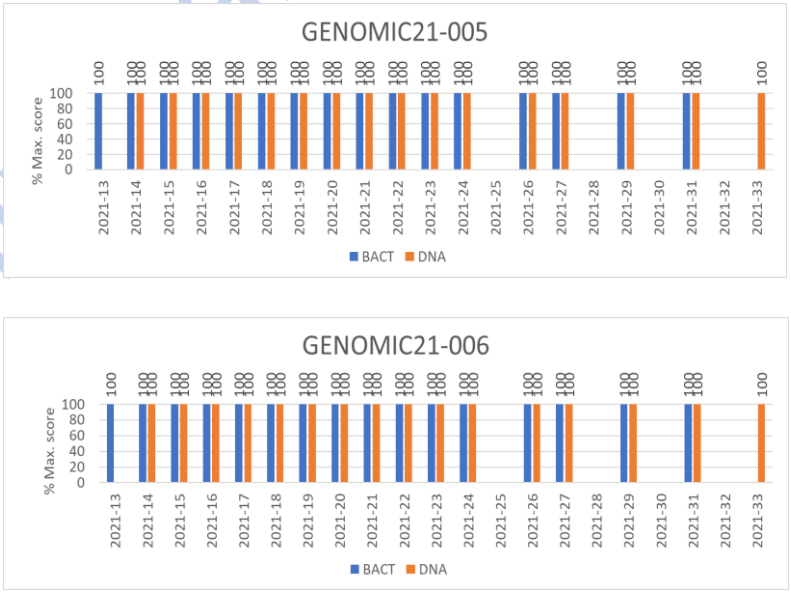


Figure 13. Performance of participants regarding the identification of chromosomal mutations conferring AMR in BACT and DNA samples, expressed as percent of the maximum score. Top: strain GENOMIC21-005, Bottom: GENOMIC21-006.

Table 18. Maximum obtainable score per participant for each category of AMR determinants (chromosomal mutations, genes, or gene variants) and the predicted AMR phenotype, as well as the total maximum score. To evaluate the performance of the participants in more detail, individual scores were assigned to each AMR phenotype submitted, instead of scoring the entire phenotypic AMR profile submitted. Scores for the elements highlighted in yellow were blanked after re-evaluation, for details see text and Table 5.

	S. enterica		***E. coli***				***C. jejuni***					
	GENOMIC21-001		**GENOMIC21-003**		**GENOMIC21-004**		**GENOMIC21-005**			**GENOMIC21-006**		
	Gene/Gene variant	**Predicted AMR phenotype**	**Gene/Gene variant**	**Predicted AMR phenotype**	**Gene/Gene variant**	**Predicted AMR phenotype**	**Chr. mutations**	**Gene/Gene variant**	**Predicted AMR phenotype**	**Chr. mutations**	**Gene/Gene variant**	**Predicted AMR phenotype**
	aph(3')-Ia	AMP	*blaNDM-4*	AMP	*aph(3'')-Ib*	STR	A2075G	*tet(O)*	ERY	A2075G	*aph(2'')-If*	CHL
	blaTEM-1A/B/C/D	AZI	*blaTEM-1A/B/C/D*	FEP	*aph(6)-Id*	SMX			TET	T86I	*cat*	CIP
	cmlA1	CHL	*dfrA12*	FOT	*sul2*	TET					*tet(O)*	ERY
	dfrA12	COL	*sul1*	FOX	*tet(B)*						*aac(6')-aph(2'')*	GEN
	mcr-1.1	ERY	*sul3*	TAZ								TET
	mef(B)	KAN	*aadA2*, *aadA2b*	ETP								
	sul3	STR		IMI								
	tet(B)	SMX		MERO								
	aac(6')-Iaa	TET		STR								
	aadA1,ant(3'')-Ia	TMP		SMX								
	aadA2, *aadA2b*			TMP								
Max score per category	**11**	**10**	**6**	**11**	**4**	**3**	**1**	**1**	**2**	**1**	**3**	**3**
Total max. score	**21**		**17**		**7**		**4**			**7**		

Performance on AMR gene/gene variant identification, per strain

The sum of scores for AMR genes and gene variants per participant is presented in Table 19, for each strain. The collective performance of all participants for AMR gene/gene variant per strain is presented in Figure 14. For strain GENOMIC21-05 the maximum possible score was obtained, while for the other strains the rates were 83-94%. Since the number of expected genes for each strain is different, it is presumably more likely to achieve a higher performance for strains with fewer expected AMR genes. For example, for strain GENOMIC21-005 there is only one expected gene, i.e. the maximum score per participant is 1 (Table 20). The individual performance of each participant in the identification of AMR genes is presented in Figure 15 for each strain (graphs A-E), for the average of the two strains for *E. coli* and *C. jejuni* (F and G) as well as for the average performance for all strains (H).

Table 19. Sum of scores per participant for AMR genes and gene variants, for each strain.

		Max score	2021-13	2021-14	2021-15	2021-16	2021-17	2021-18	2021-19	2021-20	2021-21	2021-22	2021-23	2021-24	2021-25	2021-26	2021-27	2021-28	2021-29	2021-30	2021-31	2021-32	2021-33
GENOMIC21-001	BACT	**11**	11	8	11	11	11	11	11	11	6	11	11	11		10	11	10	10	9	8		10
	DNA	**11**		8	11	11	11	11	11	11	6	11	11	11		10	11	11	11	9	8		10
GENOMIC21-003	BACT	**6**	6	5		6	6	6	4	6	4	6	6	6		6	6	6	6	6	5		6
	DNA	**6**		5		6	6	6	4	6	4	6	6	6		6	6	6	6	6	5		6
GENOMIC21-004	BACT	**4**	4	2		4	4	4	3	4	2	4	4	4		4	4	4	4	4	2		4
	DNA	**4**		2		4	4	4	3	4	2	0	4	4		4	4	4	4		2		4
GENOMIC21-005	BACT	**1**	1	1	1	1	1	1	1	1	1	1	1	1		1	1		1		1		
	DNA	**1**		1	1	1	1	1	1	1	1	1	1	1		1	1		1		1		1
GENOMIC21-006	BACT	**3**	3	2	3	3	3	3	3	3	2	3	3	3		3	3		2		2		
	DNA	**3**		2	3	3	3	3	3	3	2	3	3	3		3	3		3		2		3

Table 20. Overview of the maximum score per participant and the maximum score for all participants for AMR genes and the number of participants that signed up for each material.

Strain	Material	Max. score per participant	Number of participants	Max score for all participants
GENOMIC21-001	BACT	11	19	209
	DNA	11	18	198
GENOMIC21-003	BACT	6	18	108
	DNA	6	17	102
GENOMIC21-004	BACT	4	18	72
	DNA	4	16	64
GENOMIC21-005	BACT	1	16	16
	DNA	1	16	16
GENOMIC21-006	BACT	3	16	48
	DNA	3	16	48

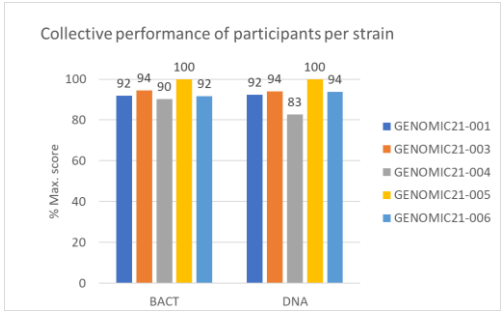


Figure 14. Collective performace of participants on the identification of all expected AMR genes, per strain, for BACT and DNA samples.

Figure 15. Performance of participants regarding the identification of AMR genes or gene variants in BACT and DNA samples, expressed as percent of the maximum score: (A) Strain GENOMIC21-001, (B) Strain GENOMIC21-003, (C) Strain GENOMIC21-004, (D) Strain GENOMIC21-005, (E) Strain GENOMIC21-006, (F): Average for E. coli, (G) Average for C. jejuni, (H) Average for all strains.

Performance on predicted AMR phenotype, per strain

The sum of scores for predicted AMR phenotype per participant is presented in Table 21, for each strain. The collective performance of all participants for predicted AMR phenotype per strain is presented in Figure 16. For strain GENOMIC21-06 the collective performance was lower (52%) compared to the other strains (79-83%). The number of expected predicted AMR phenotypes for each strain is different, it is therefore presumably more likely to achieve a better performance for strains with fewer expected AMR phenotypes (Table 22). However, this was not observed in the submitted data. The individual performance of each participant in the reporting of predicted AMR phenotype is presented in Figure 17 for each strain (graphs A-E), for the average of *E. coli* and *C. jejuni* (F and G) as well as the average performance for all strains (H).

Table 21. Sum of scores per participant for predicted AMR phenotype, for each strain. Crossed cells: lab did not participate.

		Max score	2021-13	2021-14	2021-15	2021-16	2021-17	2021-18	2021-19	2021-20	2021-21	2021-22	2021-23	2021-24	2021-25	2021-26	2021-27	2021-28	2021-29	2021-30	2021-31	2021-32	2021-33
GENOMIC21-001	BACT	**10**	9	7	10	10	10	10	10	8	0	9	10	7		10	7	10	0	7	7		9
	DNA	**10**		6	10	10	10	10	10	8	0	9	10	7		10	7	10	0	7	7		9
GENOMIC21-003	BACT	**11**	10	10		11	11	10	11	11	0	11	11	10		11	10	10	0	9	10		9
	DNA	**11**		10		11	11	10	11	11	0	11	11	10		11	10	10	0	9	10		9
GENOMIC21-004	BACT	**3**	3	2		3	3	3	3	3	0	3	3	2		3	2	3	0	2	2		3
	DNA	**3**		2		3	3	3	3	3	0	0	3	2		3	2	3	0		2		3
GENOMIC21-005	BACT	**2**	2	2	2	2	2	2	2	2	0	2	2	0		2	2		0		2		
	DNA	**2**		2	2	2	2	2	2	2	0	2	2	0		2	2		0		2		2
GENOMIC21-006	BACT	**3**	2	2	3	2	2	0	2	2	0	2	3	0		3	1		0		1		
	DNA	**3**		2	3	2	2	0	2	2	0	2	3	0		3	1		0		1		2

Table 22. Overview of the maximum score per participant and the maximum score for all participants for AMR phenotype and the number of participants that signed up for each material.

Strain	Material	Max. score per participant	Nr. of participants	Max score for all participants
GENOMIC21-001	BACT	10	19	190
	DNA	10	18	180
GENOMIC21-003	BACT	11	18	198
	DNA	11	17	187
GENOMIC21-004	BACT	3	18	54
	DNA	3	16	48
GENOMIC21-005	BACT	2	16	32
	DNA	2	16	32
GENOMIC21-006	BACT	3	16	48
	DNA	3	16	48

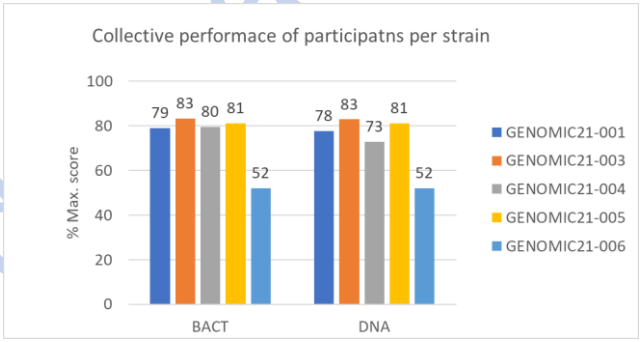
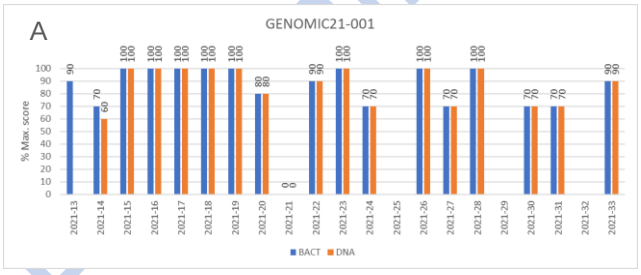
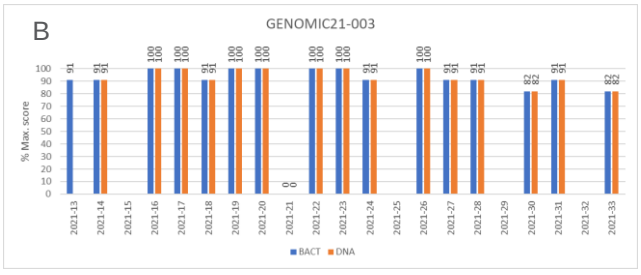
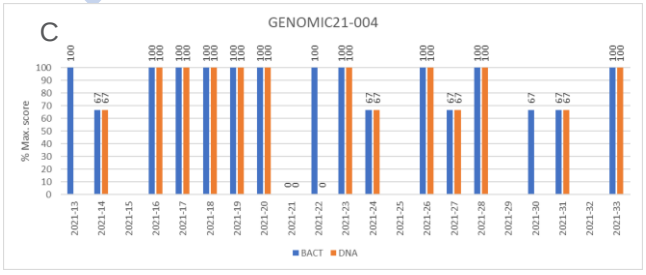
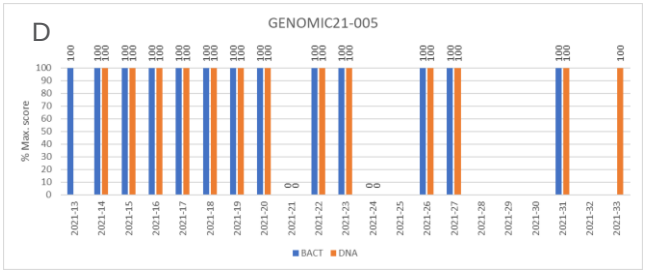


Figure 16. Collective performace of participants on the identification of all expected predicted AMR phenotypes, per strain, for BACT and DNA samples.









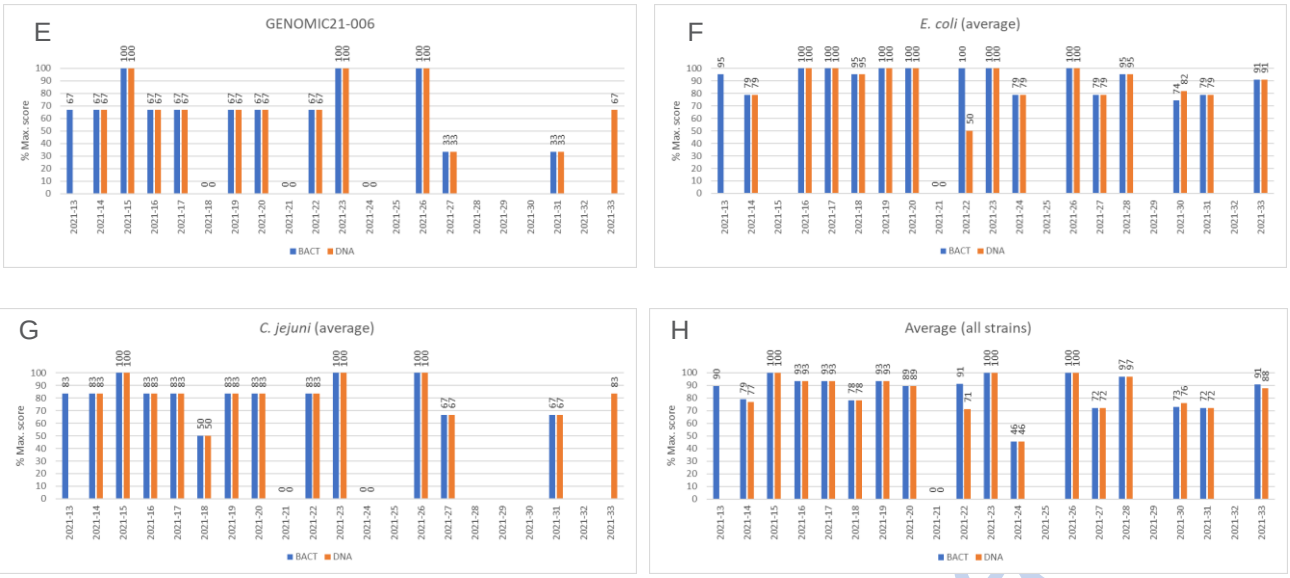


Figure 17. Performance of participants regarding the detection of predicted AMR phenotypic profile in BACT and DNA samples for strains: (A) GENOMIC21-001, (B) GENOMIC21-003, (C) GENOMIC21-004, (D) GENOMIC21-005, (E) GENOMIC21-006, (F): Average for E. coli, (G) Average for C. jejuni, (H) Average for all strains.

6. Concluding remarks

This document provides supplementary material to the participants of the Genomic PT 2021 and should be used as an aid to understand the scoring system of the AMR part of the PT and to perform self-evaluation, applying internal acceptance criteria. The present document offers a preliminary overview of the findings of the Genomic PT 2021 and should be used for the purpose stated above. The Genomic PT 2021 data together with data from Genomic PT 2020 will be presented in a journal publication (manuscript currently in preparation).