European Commission

Service contract for the provision of EU networking and support for public health reference laboratory functions for antimicrobial resistance in *Salmonella* species and *Campylobacter* species in human samples
SC 2019 74 09

Deliverable T1.17.2

Report on the second annual inter-laboratory ring-trial of bioinformatics pipelines for *Salmonella* and *Campylobacter*

Version n°: 2

Written by Statens Serum Institut
January – 2024

STATENS SERUM INSTITUT

DTU

FWD AMR · RefLabCap

This report was produced under the EU Third Health Programme 2014-2020 under a service contract with the Consumers, Health, Agriculture and Food Executive Agency (Chafea) acting under the mandate from the European Commission. From 1 April 2021, the European Health and Digital Executive Agency (HaDEA) took over all contractual obligations from Chafea. The information and views set out in this report are those of the authors and do not necessarily reflect the official opinion of the Commission/Executive Agency. The Commission/Executive Agency do not guarantee the accuracy of the data included in this study. Neither the Commission/Executive Agency nor any person acting on the Commission's/Executive Agency's behalf may be held responsible for the use which may be made of the information contained therein.

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Report on the second annual inter-laboratory ring-trial of bioinformatics pipelines for *Salmonella* and *Campylobacter*

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1. Background

This report presents the organisation and performance in the second *in silico* inter-laboratory ring trial of bioinformatics pipelines for prediction of AMR genes in antimicrobial resistant *Salmonella* and *Campylobacter* (RingTrial2-WGS-AMR, hereafter referred to as RingTrial2, or RT2), the second out of three planned ring trials, organized by Statens Serum Institut (SSI) in the FWD AMR-RefLabCap project in years 2022-24.

The second ring trial was organised according to the work plan (Deliverable T1.7), as well as using information from the first ring trial outcome. The overall aim of this ring trial was to compare the outcome of different databases, tools and bioinformatic pipelines used by the participants in order to determine antimicrobial resistance genes and point mutations in the provided DNA sequences. The participants were encouraged to follow the guidelines in the FWD AMR-RefLabCap WGS protocol (https://www.fwdamr-reflabcap.eu/resources/reflabcap-protocols-and-guidelines) concerning recommendations for prediction of resistance traits. Participation in the RingTrial2 will enable the laboratories to identify strengths and weaknesses in their analytical setup and implement improvements, when needed. Furthermore, the outcome will be discussed with the network at a webinar.

DNA sequences (paired end Illumina reads and SPAdes assemblies) from five *Salmonella* and five *Campylobacter* strains were included in this ring trial. Thirty-six participants from 29 countries (including 10 “priority countries”) accepted the invitation and have submitted results.

2. Materials and methods

2.1. Sequence selection

The strain sequences used in this RingTrial2 represent a wide array of antimicrobial resistance markers and were selected from the SSI strain collection. The genotypic and phenotypic antimicrobial resistance features of each strain are shown in Table 1 and Table 2.

Table 1. Genotypic and phenotypic characteristics of Salmonella strains selected for the RingTrial2

Strain	TRING2S-1	TRING2S-2	TRING2S-4	TRING2S-7	TRING2S-10
Serotype	Saintpaul	Meleagridis	Typhimurium	Newport	Monophasic Typhimurium
ST	50	463	19	132	34
Genes[A]	*aac(3)-IId, aadA2, aph(3’)-Ia, aph(3’’)-Ib, aph(6)-Id, arr-2, blaTEM-1, dfrA14, floR, lnu(F), mph(A), qnrS1, sul2, tet(A)*	*aac(3)-IId, aac(6’)-Ib-cr5, aadA16, aph(3’’)-Ib, aph(6)-Id, arr-3, blaTEM-1, catA2, dfrA27, floR, fosA7.4, mph(A), qnrB6, sul1, sul2, tet(A)*	*aadA2, ant(2’’)-Ia, blaCTX-M-9, catA1, dfrA16, qnrA1, sul1, tet(A)*	*aadA2, blaCARB-2, dfrA1, floR, mph(A), qnrA1, sul1, tet(A)*	*aac(3)-IIg, aac(6’)-Ib3, aac(6’)-IIc, aadA2, aph(3’)-Ia, aph(3’’)-Ib, aph(6)-Id, arr, blaSHV-12, blaTEM-1, dfrA19, ere(A), qnrB2, sul1, sul2, tet(B), tet(D)*
PMs[A]	*gyrA* S83Y	None	None	None	None
NWT Phenotypes[B]	AMP, AZI, CHL, CIP, GEN, NAL, SME, TET, TIG, TRI	AMP, AZI, CHL, CIP, SME, TET, TRI	AMP, CTA, CHL, CIP, GEN, SME, TET, TRI	AMP, AZI, CHL, CIP, SME, TET, TRI	AMP, CTA, CTZ, CIP, GEN, SME, TET, TRI

A According to AMRFinderPlus

B Abbreviations of antimicrobials: AMP (Ampicillin), AZI (Azithromycin), Cefotaxime (CTA), Ceftazidime (CTZ), CHL (Chloramphenicol), CIP (Ciprofloxacin), GEN (Gentamicin), NAL (Nalidixic acid), SME (Sulfamethoxazole), TEM (Temocilin), TET (Tetracycline), TIG (Tigecycline), TRI (Trimethoprim) . Abbreviations used are based on EUCAST system : https://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Disk_test_documents/Disk_abbreviations/EUCAST_system_for_antimicrobial_abbreviations.pdf

Please, note that when reporting to TESSy, different letter codes are applied for certain antimicrobials: AZM (Azithromycin), CTX (Cefotaxime), CAZ (Ceftazidime), SMX (Sulfamethoxazole), TCY (Tetracycline), TGC (Tigecycline), TMP (Trimethoprim).

Table 2. Genotypic and phenotypic characteristics of Campylobacter strains selected for the RingTrial2

Strain	TRING2C-1	TRING2C-4	TRING2C-7	TRING2C-9	TRING2C-10
Species	*C. coli*	*C. jejuni*	*C. jejuni*	*C. jejuni*	*C. coli*
ST	9263	257	7433	572	12073
Genes[A]	*aad9, aadE, aadE-Cc, blaOXA-193, lnuC, tetO*	*blaOXA-461*	*aad9, aph(2'')-If, aph(3')-IIIa, blaOXA-193, catA13, tetO*	*aadE, aph(3')-IIIa, blaOXA-193, sat4*	*aad9, aadE, aph(3')-IIIa, blaOXA-193, catA, ermB, sat4, tetO*
PMs[A]	*gyrA* T86I	None	*50S* L22 A103V, *gyrA* T86I	*gyrA* T86I	*gyrA* T86I, *rpsL* K43R
NWT Phenotypes[B]	CIP, ERY, NAL, STR, TET	None	CHL, CIP, ERT, GEN, TET	CIP, TET	CHL, CIP, ERT, ERY, TET

A According to AMRFinderPlus
B Abbreviations of antimicrobials: CHL (Chloramphenicol), CIP (Ciprofloxacin), ERT (Ertapenem), ERY (Erythromycin), GEN (Gentamicin), NAL (Nalidixic acid), STR (Streptomycin), TET (Tetracycline). Abbreviations used are based on EUCAST system : https://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Disk_test_documents/Disk_abbreviations/EUCAST_system_for_antimicrobial_abbreviations.pdf
Please note that when reporting to TESSy, different letter codes are applied for certain antimicrobials: ETP (Ertapenem), TCY (Tetracycline).

2.2. Phenotypic testing by the Ring Trial provider

The strains were phenotypically tested for antimicrobial susceptibility by determination of MIC values and subsequent classification as wild type (wt) or non-wild type (nwt) using epidemiological break point values (1). MIC determination was performed following the harmonised EU AST protocol using microbroth dilution method with EUVSEC and EUVSEC3 TREK panels from Thermo Scientific, Denmark for *Salmonella* and EUCAMP2 and EUCAMP3 panels for *Campylobacter* (https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32020D1729&from).

The *Salmonella* panels included the following antimicrobials: Amikacin (for one strain only) Ampicillin, Azithromycin, Cefepime, Cefotaxime, Cefoxitin, Ceftazidime, Chloramphenicol, Ciprofloxacin, Colistin, Ertapenem, Gentamicin, Meropenem, Nalidixic acid, Sulfamethoxazole, Temocillin, Tetracycline, Trimethoprim and Trimethoprim-sulfamethoxazole. For *Campylobacter*, the panels included Ciprofloxacin, Erythromycin, Gentamicin, Streptomycin (for one strain) and Tetracycline. The results are shown in Table 1 and Table 2. The selection of antimicrobials tested was based on the priority list of antimicrobial agents set in the harmonised EU AST protocol (1), recommended by the ECDC.

In most cases there is a correlation between the pheno- and genotypes and from the established phenotypes for the test strains it is possible to evaluate the phenotypic predictions for these antimicrobials. However, the strains included in this RingTrial also harbour genes that confer resistance towards antimicrobials that the test strains have not been tested for. This is *e.g.* the case for a lincosamide gene in *Salmonella* and beta-lactam genes in *Campylobacter*.

2.3. WGS analysis by the Ring Trial provider

DNA from *Salmonella* and *Campylobacter* strains was sequenced using paired-end Illumina sequencing. The quality of the sequences (genome size, N50, total number of contigs) was checked with an in-house QC pipeline (https://github.com/ssi-dk/bifrost) for raw reads and BioNumerics for assemblies.

Salmonella serotypes were determined using Enterobase and SeqSero (https://github.com/denglab/SeqSero), as well as in-house developed scripts detecting the subspecies and genetic marker implicating the *d*-Tartrate reaction for distinguishing *S* Paratyphi B var. L(+) tartrate+ (var. Java) from *S* Paratyphi B.

For *Campylobacter* species identification, Kraken was used (https://github.com/DerrickWood/kraken). MLST calling was performed with ARIBA (https://github.com/sanger-pathogens/ariba) using the typing schemes from the PubMLST database.

The sequences were analysed by the Ring Trial provider in April 2023 for the presence of antimicrobial resistance genes and point mutations by querying two different databases: ResFinder and AMRFinderPlus. The results obtained with the two approaches shown in Table 3, will be referred as “reference datasets” in the report.

Table 3. Tools and databases used in provider’s reference data sets for Salmonella and Campylobacter

	AMR gene detection			**Point mutation identification**		
	Database	**Tool**	**Input**	**Database**	**Tool**	**Input**
Ref_Res	ResFinder	ResFinder	Raw reads (fastq)	ResFinder	PointFinder	Raw reads (fastq)
Ref_AMR	AMRFinderPlus	AMRFinderPlus	SPAdes assembly (fasta protein)	AMRFinderPlus	AMRFinderPlus	SPAdes assembly (fasta nucleotide)

In the result analysis, each reference dataset was compared to genes and point mutations reported by the participants using the same database or a combination of databases.

Sequences from all strains, in the form of paired-end Illumina reads (fastq files) or SPAdes assemblies (fasta files) were shared with all participants via an FTP server.

2.4. SurveyXact reporting scheme

The reporting platform was developed in the SurveyXact survey tool (https://www.survey-xact.dk).

The reporting scheme consisted of two parts. The first part included questions about tools and databases used to identify the sequence type (ST), AMR genes, point mutations (PMs), as well as the serotype and species for *Salmonella* and *Campylobacter*, respectively. Furthermore, questions in this part included identity and coverage cut-offs used for identifying genes and point mutations, as well as an additional question for participants which reported that they had used more than one database. The second part was for reporting AMR genes and point mutations. It was possible to select multiple genes from a list of genes in alphabetical order. It was also possible to report a gene in a free text field, in case it was not present on the default list. For reporting of point mutations, the participants were asked to type the detected mutations in text boxes.

All participants received individual links to the reporting form, where it was possible to report results for one or both pathogens. The time given for reporting of the results was one month after sharing the sequences.

Twenty-three participants reported results for both *Salmonella* and *Campylobacter*, six laboratories for *Salmonella* only and six for *Campylobacter* only. The participating countries were Austria, Belgium, Bulgaria, Croatia, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg,

Malta, Netherlands, Norway, Poland, Portugal, Romania, Serbia, Slovenia, Spain and Sweden. The participating laboratories were randomly assigned codes and these codes were used for identification of laboratories in the ring trial.

3. Salmonella results

3.1. AMR gene and PMs detection methods used

3.1.1. Tools and databases used for AMR gene detection

All 29 participants reported the applied tools, databases, types of files used as inputs, thresholds for sequence coverage and sequence identity for AMR gene detection, as well as how they reported the genes. Overall, 25 unique combinations of tools/databases/inputs/thresholds/gene reporting strategies were used by 29 participants (Table S 1).

The most commonly used tool was ResFinder, followed by AMRFinderPlus. ResFinder was used by 23 participants and AMRFinderPlus by 13 participants. The remaining seven tools were used by up to two participants each (Figure 1).

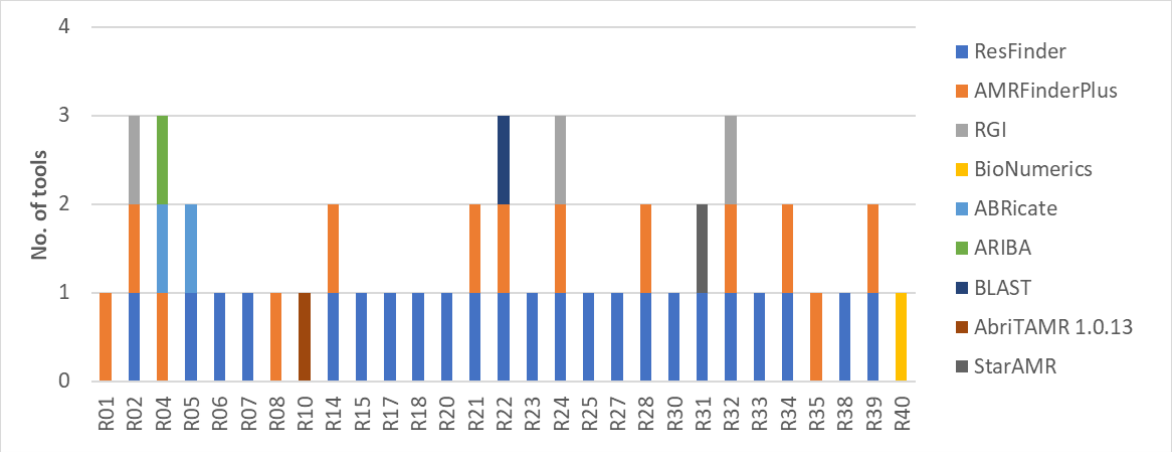

Figure 1. An overview of tools used by 29 participants for AMR gene detection in Salmonella

The ResFinder database was used by 25 participants and the AMRFinderPlus database was used by 13 participants. The CARD database was used by four participants, and the AbriTAMR 1.0.13 and BioNumerics were used by one participant each. It is assumed by the provider that the participant who reported the AbriTAMR 1.0.13 tool as the database used in fact the AMRFinderPlus database, which is the default for this tool. The participants, who used more than one database, also indicated how they reported AMR genes. Four participants reported a consensus list of genes (common genes present in all databases used), five participants reported a subset of genes based on experience/knowledge/literature, two participants reported all genes from all databases, and one participant indicated the reporting of consensus and subset of genes that were only in one of databases (R28). Additionally, participant R06 indicated that literature was used where necessary, and participant R24 that they report genes that are present in at least two databases, a sort of a voting system (Figure 2).

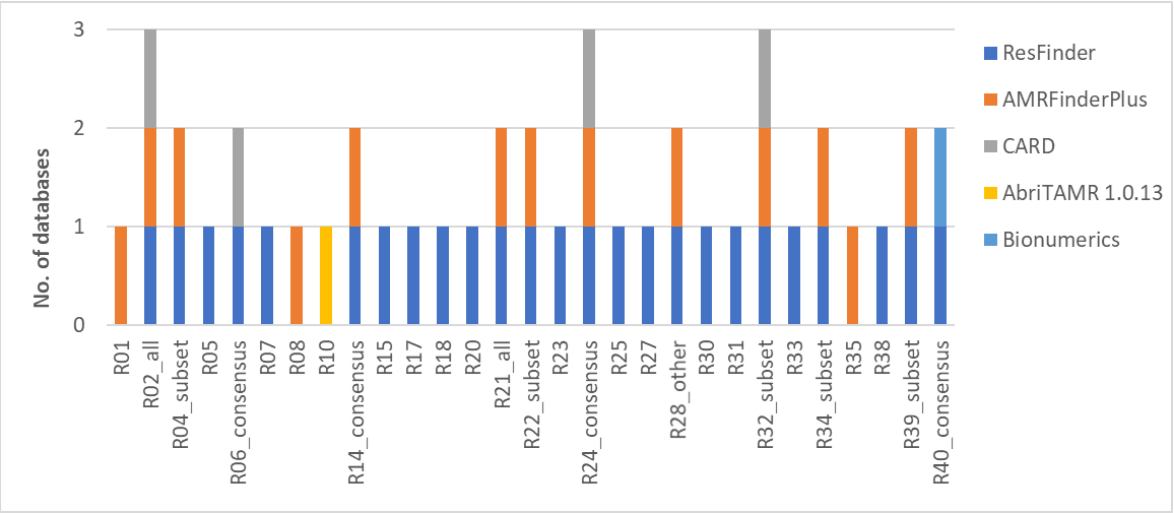

Figure 2. An overview of databases used by 29 participants for AMR gene detection in Salmonella. The horizontal labels indicate the participant ID and how they reported AMR genes in cases when more than one database was used: consensus – common genes present in all databases used, subset – based on experience/knowledge/literature, all – genes from all databases. other – other strategy. It is assumed by the provider that participant R10, who reported the tool AbriTAMR 1.0.13 as the database, used the AMRFinderPlus database, which is the default for this tool.

3.1.2. Tools and databases used for point mutation detection

All 29 participants reported the tools, the databases, and the inputs that they used for detection of point mutations and also reported what approach was applied in cases when more than one database was used. Overall, 15 unique combinations of tools/databases/inputs/reporting strategies were used by the 29 participants (Table S 2).

PointFinder was the preferred tool, being used by 23 participants, either alone or in combination with another tool. AMRFinderPlus was the second most common tool and was used by 13 participants (Figure 3).

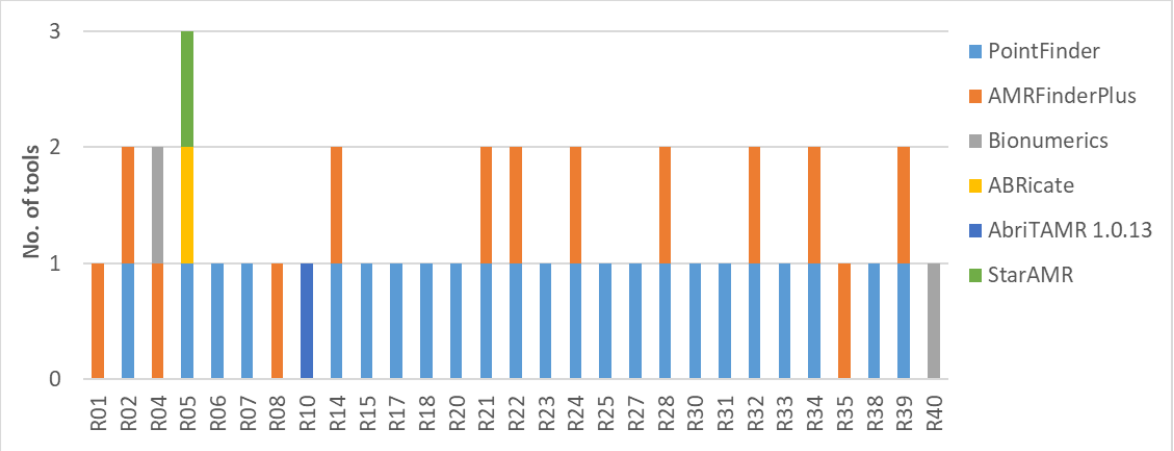

Figure 3. An overview of tools used by 29 participants for point mutations detection in Salmonella

Twenty participants used only one database and nine participants used two databases (ResFinder and AMRFinderPlus). The participants that used more than one database also indicated how they had reported the point mutations (Figure 4).

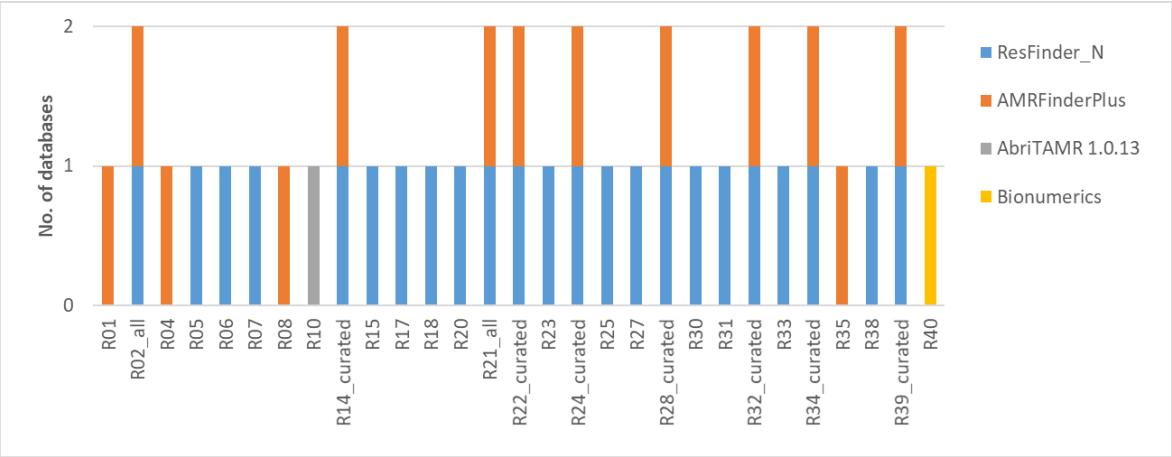

Figure 4. An overview of databases used by 29 participants for point mutations detection in Salmonella. The horizontal labels indicate the participant ID and how they reported point mutations in cases when more than one database was used: all reported all point mutations from all databases without curating, curated - curated the point mutations from all databases for duplicates. It is assumed by the provider that participant R10, who reported the tool AbriTAMR 1.0.13 as the database, used the AMRFinderPlus database, which is the default for this tool.

3.2. Serotypes and STs reported

3.2.1. Serotyping methods and serotypes

Twenty participants used only one tool/software for *Salmonella* serotyping, and nine participants used a combination of two or three tools/softwares. The most commonly used tool was SeqSero2, used by 22 participants (Figure 5).

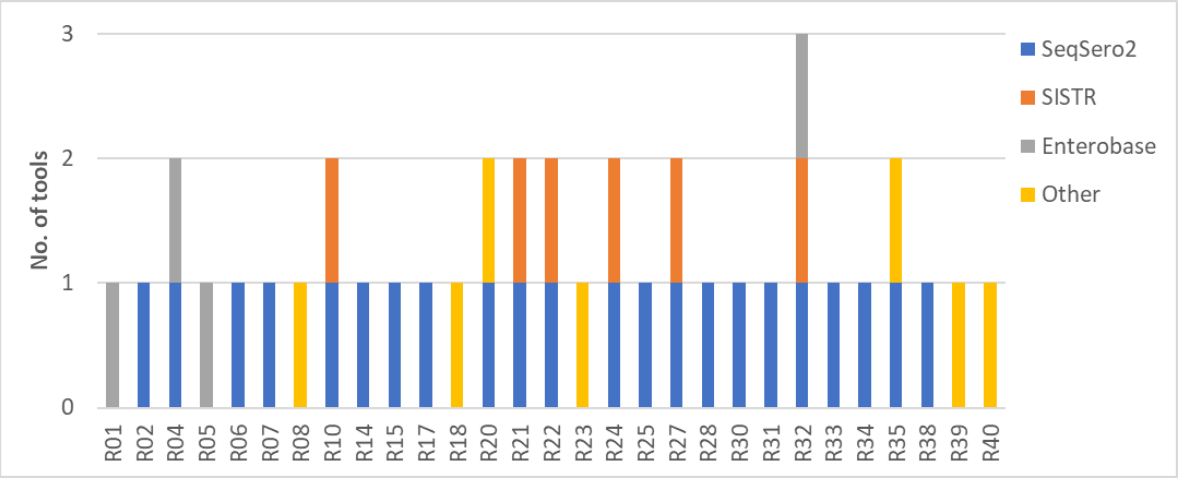

Figure 5. An overview of the tools/softwares used by 29 participants for Salmonella serotyping

All 29 participants reported serotypes for strains TRING2S-1, TRING2S-2 and TRING2S-10 and all but one participant reported serotypes for strains TRING2S-4 and TRING2S-7. The reported serotypes from 29 participants for the test samples are presented in Table 4. In general the participants reported serotypes that were in accordance with the serotypes identified by the ring trial provider.

Table 4. Reported Salmonella serotypes and concordance with RT provider results.

	TRING2S-1	TRING2S-2	TRING2S-4	TRING2S-7	TRING2S-10
Serotype	Saintpaul	Meleagridis	Typhimurium	Newport	4,5,12:i:-, monophasic Typhimurium
Concording results	27	28	27	27	27
Non-concording results	2	1	1	1	2
No serotype reported			1	1	

In total, seven non-concording results were reported, of which three were reported by the same participant. Two participants incorrectly reported the monophasic Typhimurium strain **TRING2S-10** as S. Typhimurium (Table S 5).

3.2.2. MLST methods and STs

All 29 participants reported which schemes/tools they used for ST identification in *Salmonella*. Thirteen participants used the MLST2.0 scheme that is available in CGE tools (https://cge.food.dtu.dk/services/MLST), five participants used the Enterobase MLST scheme, and four participants used the Tsemann MLST scheme. The remaining seven participants indicated that they used other schemes/tools: Ridom SeqSphere+ (R01, R08, R22), Bifrost using Enterobase scheme (R04), stringMLST/PubMLST (R33), staramr tool using senterica_achtman_2 scheme on galaxy platform (R39), and BioNumerics 8.1 (R40). All participants reported correct ST for all three strains (Table S 6).

3.3. AMR genes and PMs reported for *Salmonella* strains

The genes identified by the ring trial provider using two different tools and databases, namely Res_Ref and AMR_Ref, and the genes identified by the participants are presented for each strain in the following paragraphs. The letter “X” indicates the detection of a specific gene.

The results from the participants and the ring trial provider’s reference datasets are divided into three categories based on which database was used. The green (ResFinder / PointFinder) category indicates laboratories that only used the ResFinder / PointFinder database (2)(3). Participants that used the AMRFinderPlus database (4), either alone or with ResFinder, are grouped in the blue category (AMRFinderPlus +/- ResFinder). When relevant, the third, yellow, category was applied, grouping the laboratories that used CARD (5), either alone or in combination with any other databases. In cases where a participant used a database different from ResFinder, AMRFinderPlus or CARD, it was marked with an asterisk in the tables and the database name was stated in the table footer.

For each gene and PM table, the concordance of the reported results among the participants was calculated. This number is expressed as a percentage of the total number of participants that reported the same genes or point mutations for a given DNA sequence. When possible, explanations of observed discrepancies between reference datasets and participant’s results or between databases were explained.

3.3.1. Strain TRING2S-1

Differences observed between the two reference datasets, Res_Ref and AMR_Ref, are summarised in Table 5, along with suggested explanations for the discrepancies.

Table 5. Suggested explanation of differences between reference datasets Res_Ref and AMR_Ref from Table 6, observed in gene reporting from Salmonella strain TRING2S-1. Grey shading indicates in which database the gene is present.

Gene	Res _Ref	AMR_ Ref	Suggested explanation	Reference/comment
aac(6')-Iaa			Gene absent from the AMRFinderPlus database. Does not contribute to aminoglycoside resistance in *Salmonella*.	(6)
aadA2			Highest similarity to gene *aadA2* (99,62%) in AMRFinderPlus.	Genes *aadA2* and *aadA17* are 95% similar
aadA17			Highest similarity to gene *aadA17* (98,86%) in ResFinder	
arr-3			Detected in ResFinder only when reads were used (mapped by KMA)	
blaTEM-1			Gene *blaTEM-1* present in AMRFinderPlus database	
blaTEM-1B			Variant *blaTEM-1B* present in ResFinder database	

The gene *mphA* was reported by all participants, whereas genes *blaTEM-1 / blaTEM-1B*, *dfrA*, *qnrS1* and *sul2* were reported by 97% (28/29) of laboratories (Table 6). The gene reported with the least concordance was *arr-3* (31%). In the reference datasets, this gene was detected only when reads were used in ResFinder, suggesting that when an assembly was used, the gene could have been split into contigs, but mapping of the reads allowed for identification of *arr-3*. Similarly, all 7 out of 13 participants that reported using ResFinder, used reads as input. Two of those participants, however, used assemblies as input additionally and it is unknown for the provider whether these participants reported read- or assembly- based results.

Table 6. AMR genes reported in Salmonella strain TRING2S-1. Reference datasets, Res_Ref and AMR_Ref, are shaded grey. Participants are grouped based on database(s) used: Green – ResFinder, Blue – AMRFinderPlus with or without ResFinder, Yellow – CARD with or without any other database. Percentage concordance is based on following scale: darkest orange colour: 100% concordance among participants, lighter orange colour: 90-99% concordance, lightest orange colour: 80-89% concordance. Concordance lower than 80% is without colour.

	ResFinder														AMRFinderPlus +/- ResFinder											CARD +/- others						
	Res_Ref	R15	R23	R25	R17	R30	R31	R27	R07	R33	R05	R18	R20	R38	AMR_Ref	R08	R35	R01	R10	R14	R04	R28	R21	R34	R39	R06	R02	R24	R32	R22	R40	% concordance
ResFinder																																
AMRFinderPlus																					**											
CARD																																
Other database																														*	**	
aac(3)-IId	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X	X	X	93
aac(6')-Iaa	X	X	X	X	X	X		X	X	X		X	X	X									X	X			X	X			X	55
aadA2			X												X	X	X	X	X	X	X	X	X		X		X	X	X	X		
aadA17	X	X	X		X	X		X	X	X	X	X	X	X										X				X					83
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		90
aph(3'')-Ib	X	X	X	X		X		X				X	X	X	X					X	X	X	X	X	X		X	X	X	X		62
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	X	X	X		90
arr-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		93
arr-3	X	X	X		X	X		X		X				X									X				X					31
blaTEM-1															X	X	X	X	X	X		X	X	X				X	X			
blaTEM-1B	X	X	X	X	X	X		X	X	X	X	X	X	X							X		X		X	X	X			X	X	97
dfrA14	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	97
floR	X	X	X		X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X	X	X	90
lnu(F)	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		93
mph(A)	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	100
qnrS1	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	97
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	97
tet(A)	X	X	X	X	X	X		X		X		X	X	X	X				X	X	X	X	X	X	X		X	X	X	X	X	76

* GenBank
** BioNumerics 8.1

The *gyrA* point mutation was reported by both reference datasets, as well as by all participants (Table 7). Two participants did not specify which amino acid substitution was detected, but only indicated the gene.

Table 7. Point mutations (PMs) reported in Salmonella strain TRING2S-1. Reference datasets, Res_Ref and AMR_Ref, are shaded grey. Participants are grouped based on database(s) used : Green – PointFinder, Blue – AMRFinderPlus with or without PointFinder, Yellow (called "O" for "other" – other database(s) (specified below the table). Percentage concordance is based on following scale : darkest orange colour : 100% concordance among participants, lighter orange colour : 90-99% concordance, lightest orange colour : 80-89% concordance. Concordance lower than 80% is without colour.

	PointFinder															AMRFinderPlus +/- PointFinder														O	
	Res_Ref	R15	R23	R25	R30	R31	R27	R07	R33	R05	R18	R20	R06	R38		AMR_Ref	R08	R04	R35	R01	R10	R14	R02	R17	R24	R32	R21	R22	R34	R39	R40	% concordance
PointFinder																															
AMRFinderPlus																		*													
Other																														*	
gyrA								X						X																	
gyrA S83Y	X	X	X	X	X	X	X		X	X	X	X	X			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	100

* BioNumerics 8.1

3.3.2. Strain TRING2S-2

Differences observed between the two reference datasets, Res_Ref and AMR_Ref, are summarised in Table 8, along with suggested explanations for the discrepancies.

Table 8. Suggested explanation of differences between reference datasets Res_Ref and AMR_Ref from Table 9, observed in gene reporting from Salmonella strain TRING2S-2. Grey shading indicates in which database the gene was present.

Gene	Res_Ref	AMR_Ref	Suggested explanation	Reference/ comment
aac(6')-Ib-cr			This gene is present only in the ResFinder database.	
aac(6')-Ib-cr5			This specific gene variant is present only in AMRFinderPlus database.	
aac(6')-Iaa			Gene absent from the AMRFinderPlus database. Does not contribute to aminoglycoside resistance in *Salmonella*.	(6)
blaTEM-1			Gene *blaTEM-1* present in AMRFinderPlus database	
blaTEM-1B			Variant *blaTEM-1B* present in ResFinder database	
fosA7			Variant *fosA7* present in ResFinder database	
fosA7.4			Variant *fosA7.4* present in AMRFinderPlus database	

In strain TRING2S-2, the concordance of gene reporting among participants was quite high, as twelve out of seventeen genes were reported by between 90 and 100% participants (Table 9). Gene *aac(6')-Iaa* was reported by approximately half of the participants and the remaining four genes were reported by over 80% participants.

Table 9. AMR Genes reported in Salmonella strain TRING2S-2. Reference datasets, Res_Ref and AMR_Ref, are shaded grey. Participants are grouped based on database(s) used : Green – ResFinder, Blue – AMRFinderPlus with or without ResFinder, Yellow – CARD with or without any other database. Percentage concordance is based on following scale : darkest orange colour : 100% concordance among participants, lighter orange colour : 90-99% concordance, lightest orange colour : 80-89% concordance. Concordance lower than 80% is without colour.

	ResFinder															AMRFinderPlus +/- ResFinder												CARD +/- others						
	Res_Ref	R15	R23	R25	R17	R30	R31	R27	R07	R33	R05	R18	R20	R38	AMR_Ref	R08	R35	R01	R10	R14	R04	R28	R21	R34	R39	R06	R02	R24	R32	R22	R40	% concordance
ResFinder																																	
AMRFinderPlus																					**												
CARD																																	
Other database																														*	**		
aac(3)-IId	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X	X	X	93
aac(6')-Ib-cr	X	X	X	X	X	X		X	X	X	X	X	X	X						X	X		X	X	X	X	X	X			X	
aac(6')-Ib-cr5															X	X	X	X	X			X	X		X				X	X		97
aac(6')-Iaa	X	X	X		X	X		X		X		X	X	X									X	X	X		X	X			X	52
aadA16	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X	X		90
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		83
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	X	X		86
arr-3	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		93
blaTEM-1															X	X	X	X	X	X		X	X						X	X		
blaTEM-1B	X	X	X	X	X	X		X	X	X	X	X	X	X							X		X	X	X	X	X				X	X	97
catA2	X	X	X		X	X	X	X		X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		86
dfrA27	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	100
floR	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X	X	X	93
fosA7	X	X	X		X	X		X	X	X		X	X	X			X						X	X	X		X	X				
fosA7.4															X	X		X	X	X	X	X	X				X		X		X	83
mph(A)	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	100
qnrB6	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X	X	X	93
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	97
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	90
tet(A)	X	X	X	X	X	X		X	X	X	X	X	X	X	X		X		X	X	X	X	X	X	X	X	X	X	X	X	X	90

* GenBank
** BioNumerics 8.1

The point mutation parC T57S was reported only in the Res_Ref dataset, as this mutation is absent from the AMRFinderPlus database. Presence of this mutation was observed in *Salmonella* strains with very low MIC values and it is unclear whether it contributes to the resistant phenotype (7). Twenty out of twenty-nine participants reported the mutation.

Table 10. Point mutations (PMs) reported in Salmonella strain TRING2S-2. Reference dataset Res_Ref is shaded grey. Participants are grouped based on database(s) used : Green – PointFinder, Blue – AMRFinderPlus with or without PointFinder. Percentage concordance is based on following scale : darkest orange colour : 100% concordance among participants, lighter orange colour : 90-99% concordance, lightest orange colour : 80-89% concordance. Concordance lower than 80% is without colour.

	PointFinder													AMRFinderPlus + PointFinder								% concordance
	Res_Ref	R15	R23	R25	R30	R31	R27	R33	R05	R18	R20	R06	R38	R04	R14	R02	R17	R24	R21	R34	R39	
PointFinder																						
AMRFinderPlus														*								
parC T57S	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	100

* BioNumerics 8.1

3.3.3. Strain TRING2S-4

Differences observed between the two reference datasets, Res_Ref and AMR_Ref, are summarised in Table 11, along with suggested explanations for the discrepancies.

Table 11. Suggested explanation of differences between reference datasets Res_Ref and AMR_Ref from Table 12, observed in gene reporting from Salmonella strain TRING2S-4. Grey shading indicates in which database the gene was present.

Gene	Res_Ref	AMR_Ref	Suggested explanation	Reference/ comment
aac(6')-Iaa			Gene absent from the AMRFinderPlus database. Does not contribute to aminoglycoside resistance in *Salmonella*.	(6)
mcr-9			Gene absent from the AMRFinderPlus database. Was not found to confer resistance to colistin.	(8)

In strain TRING2S-4, concordance higher than 90% among the participants was achieved for six out of ten genes (Table 12). No point mutations were detected in this strain.

Table 12. AMR Genes reported in Salmonella strain TRING2S-4. Reference datasets, Res_Ref and AMR_Ref, are shaded grey. Participants are grouped based on database(s) used : Green – ResFinder, Blue – AMRFinderPlus with or without ResFinder, Yellow – CARD with or without any other database. Percentage concordance is based on following scale : darkest orange colour : 100% concordance among participants, lighter orange colour : 90-99% concordance, lightest orange colour : 80-89% concordance. Concordance lower than 80% is without colour.

	ResFinder														AMRFinderPlus +/- ResFinder														CARD +/- others						% concordance
	Res_Ref	R15	R23	R25	R17	R30	R31	R27	R07	R33	R05	R18	R20	R38	AMR_Ref	R08	R35	R01	R10	R14	R04	R28	R21	R34	R39	R06	R02	R24	R32	R22	R40	
ResFinder																																	
AMRFinderPlus																					**												
CARD																																	
Other database																														*	**		
aac(6')-Iaa	X	X	X	X	X	X		X	X	X		X	X	X								X	X	X	X	X	X	X	X		X	69	
aadA2	X	X	X		X	X		X		X	X	X		X	X		X		X	X	X	X	X	X	X		X	X	X	X			
aadA2b				X					X				X																				83
ant(2'')-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	X	97
blaCTX-M-9	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	97
catA1	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	100
dfrA16	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	93
mcr-9	X	X	X		X	X		X		X	X	X	X	X			X		X		X	X	X	X	X	X	X				X	69
qnrA1	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	97
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	97
tet(A)	X	X	X		X	X		X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X	X			83

* GenBank
** BioNumerics 8.1

3.3.4. Strain TRING2S-7

The only difference observed between the reference datasets for strain TRING2S-7 was *aac(6')-Iaa*, reported in the Res_Ref dataset and not in the AMR_Ref dataset (Table 13). This gene does not contribute to aminoglycoside resistance in *Salmonella* and is absent from the AMRFinderPlus database (6). Seven out of nine genes in this strain were detected by more than 90% of participants.

Table 13. AMR Genes reported in Salmonella strain TRING2S-7. Reference datasets, Res_Ref and AMR_Ref, are shaded grey. Participants are grouped based on database(s) used : Green – ResFinder, Blue – AMRFinderPlus with or without ResFinder, Yellow – CARD with or without any other database. Percentage concordance is based on following scale : darkest orange colour : 100% concordance among participants, lighter orange colour : 90-99% concordance, lightest orange colour : 80-89% concordance. Concordance lower than 80% is without colour.

	ResFinder														AMRFinderPlus +/- ResFinder										CARD +/- others						
	Res_Ref	R15	R23	R25	R17	R30	R31	R27	R07	R33	R05	R18	R20	R38	AMR_Ref	R08	R35	R01	R10	R14	R04	R28	R21	R34	R39	R06	R02	R24	R32	R22	R40	% concordance
ResFinder																															
AMRFinderPlus																					**										
CARD																															
Other database																													*	**	
aac(6')-Iaa	X	X	X	X	X	X		X	X	X		X	X	X								X	X	X	X	X	X	X	X		X	69
aadA2	X	X	X		X	X		X		X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		
aadA2b				X					X				X																			93
blaCARB-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	97
dfrA1	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	97
floR	X	X	X		X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X	X	X	90
mph(A)	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	100
qnrA1	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	97
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	97
tet(A)	X	X	X	X	X	X		X	X	X	X	X	X	X	X		X		X	X	X	X	X	X	X	X	X	X	X	X	X	90

* GenBank
** BioNumerics 8.1

Similarly to strain TRING2S-2, the mutation in *parC* gene was detected only in the reference dataset Res_Ref (Table 14). It was reported by 19 out of 29 participants.

Table 14. Point mutations (PMs) reported in Salmonella strain TRING2S-7. Reference dataset Res_Ref is shaded grey. Participants are grouped based on database(s) used : Green – ResFinder, Blue – AMRFinderPlus with or without ResFinder. Percentage concordance is based on following scale : darkest orange colour : 100% concordance among participants, lighter orange colour : 90-99% concordance, lightest orange colour : 80-89% concordance. Concordance lower than 80% is without colour.

	PointFinder												AMRFinderPlus + PointFinder								
	Res_Ref	R15	R23	R25	R30	R27	R33	R05	R18	R20	R06	R38	R04	R14	R02	R17	R24	R21	R34	R39	% concordance
ResFinder																					
AMRFinderPlus													*								
parC												X									
parC T57S	X	X	X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	95

* BioNumerics 8.1

3.3.5. Strain TRING2S-10

Differences observed between the two reference datasets, Res_Ref and AMR_Ref, are summarised in Table 15, along with suggested explanations for the discrepancy.

Table 15. Suggested explanation of differences between reference datasets Res_Ref and AMR_Ref from Table 16, observed in gene reporting from Salmonella strain TRING2S-10. Grey shading indicates in which database the gene was present.

Gene	Res_ Ref	AMR_ Ref	Suggested explanation	Reference/ comment
aac(3)-IIg			Variant absent from ResFinder database	
aac(6')-Ib			Variant present in ResFinder database. Even though 9 variants of this gene are present in AMRFinderPlus, the gene was not listed as a result. Instead, gene *aac(6')-Ib3*, which is 99.5% identical to *aac(6')-Ib*, was reported in AMRFinderPlus	Similarities based on ClustalW (https://www.genome.jp/tools-bin/clustalw)
aac(6')-Ib-cr			Variant present in ResFinder database. Even though 9 variants of this gene are present in AMRFinderPlus, the gene was not listed as a result. Instead, gene *aac(6')-Ib3*, which is 99.6% identical to *aac(6')-Ib-cr*, was reported in AMRFinderPlus	Similarities based on ClustalW (https://www.genome.jp/tools-bin/clustalw)
aac(6')-Iaa			Gene absent from the AMRFinderPlus database. Does not contribute to aminoglycoside resistance in *Salmonella*.	(6)
arr			ResFinder database contains 10 variants of the *arr* gene but the gene from this strain was missed by this database. AMRFinderPlus database contains 5 named alleles of this gene and 8 additional alleles that are unnamed and listed as *arr* or *arr-3* gene family. It could be speculated that the *arr* allele in this strain is novel.	
blaTEM-1			Gene *blaTEM-1* present in AMRFinderPlus database	
blaTEM-1B			Variant *blaTEM-1B* present in ResFinder database	
mcr-9			Gene absent from the AMRFinderPlus database. Was not found to confer resistance to colistin.	(8)

In strain TRING2S-10, eleven out of nineteen genes were detected by more than 90% of participants, including five genes, *aac(6')-Ib3*, *aac(6')-IIc*, *blaSHV-12*, *blaTEM-1* and *qnrB2* reported by all participants (Table 16). It is worth noting that participant R40, who used BioNumerics 8.1 database for analysis, reported discrepancy in detection of *sul2* gene depending on whether reads or assemblies were used. For this reason, the participant supplemented the analysis of this strain with analysis in ResFinder, where *sul2* was detected independently of the input used. No point mutations were detected in this strain.

Table 16. AMR Genes reported in Salmonella strain TRING2S-10. Reference datasets, Res_Ref and AMR_Ref, are shaded grey. Participants are grouped based on database(s) used : Green – ResFinder, Blue – AMRFinderPlus with or without ResFinder, Yellow – CARD with or without any other database. Percentage concordance is based on following scale : darkest orange colour : 100% concordance among participants, lighter orange colour : 90-99% concordance, lightest orange colour : 80-89% concordance. Concordance lower than 80% is without colour.

	ResFinder														AMRFinderPlus +/- ResFinder										CARD +/- others						
	Res_Ref	R15	R23	R25	R17	R30	R31	R27	R07	R33	R05	R18	R20	R38	AMR_Ref	R08	R35	R01	R10	R14	R04	R28	R21	R34	R39	R06	R02	R24	R32	R22	R40	% concordance
ResFinder																															
AMRFinderPlus																					**										
CARD																															
Other database																													*	**	
aac(3)-IIg															X	X	X	X	X	X	X	X	X				X		X	X		38
aac(6')-Ib	X					X		X		X																					
aac(6')-Ib3	X	X	X	X	X	X	X	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')-Ib-cr	X	X	X		X	X		X	X	X	X	X	X	X									X	X	X		X					100
aac(6')-IIc	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	100
aac(6')-Iaa	X	X	X	X	X	X		X	X	X		X	X	X									X	X	X	X		X	X		X	62
aadA2	X	X	X		X	X		X		X	X	X		X	X	X	X	X	X	X	X	X	X	X	X		X	X	X	X		
aadA2b				X					X				X																			90
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		90
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		83
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	X	X		83
arr															X	X	X	X	X		X	X	X		X		X		X	X		38
blaSHV-12	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	100
blaTEM-1															X	X	X	X	X	X		X	X						X	X			
blaTEM-1B	X	X	X	X	X	X	X	X	X	X	X	X	X	X							X		X	X	X	X	X			X	X	100
dfrA19	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X	X	X	97
ere(A)	X	X	X	X	X	X		X				X	X	X	X					X		X	X	X	X	X	X	X	X	X		66
mcr-9	X	X	X		X	X	X	X	X	X	X	X	X	X			X		X	X	X	X	X	X	X	X	X				X	79
qnrB2	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	100
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	97
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	93
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	97
tet(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	97

* GenBank
** BioNumerics 8.1

4. Campylobacter results

4.1. AMR gene and PMs detection methods used

4.1.1. Tools and databases used for gene detection

All 29 participants reported the applied tools, databases, types of files used as inputs, thresholds for sequence coverage and sequence identity for AMR gene detection, as well as how they reported the genes. Overall, 24 unique combinations of tools/databases/inputs/thresholds/gene reporting strategies were used by 29 participants (Table S 3).

The most commonly used tool was ResFinder, followed by AMRFinderPlus: ResFinder was used by 24 participants and AMRFinderPlus by 12 participants. Remaining seven tools were used by 1-3 participant each (Figure 6).

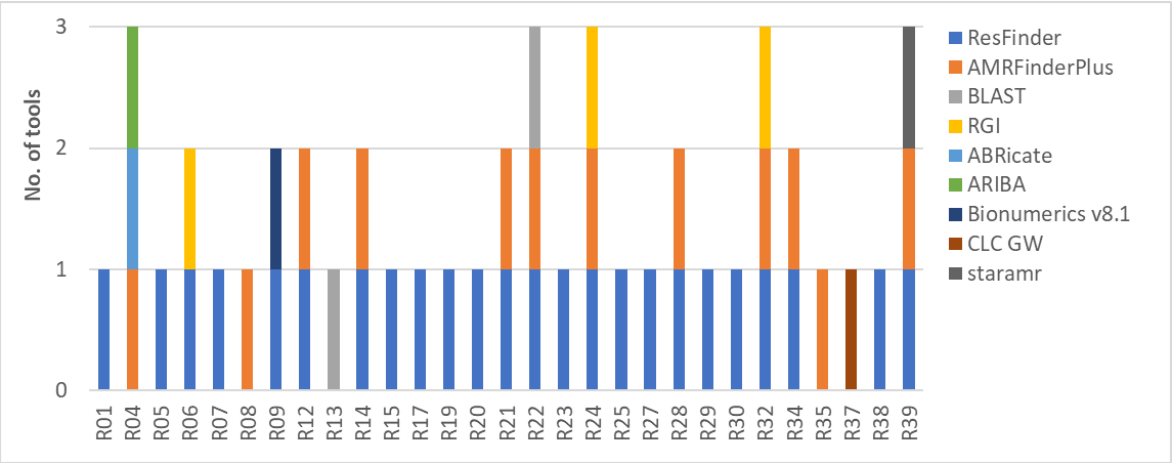

Figure 6. An overview of tools used by 29 participants for AMR gene detection in Campylobacter

The ResFinder database was used by 26 participants, the AMRFinderPlus database by 12 participants and the CARD database by four participants. Remaining databases were used by one participant each (Figure 7). The participants which used more than one database also indicated how they reported AMR genes. Three participants reported a consensus list of genes (common genes present in all databases used), seven participants reported a subset of genes based on experience/knowledge/literature, one participant reported all genes from all databases and one participant indicated reporting a consensus and subset of genes from one of databases (R28). Additionally, participant R06 indicated that literature was used where necessary, and participant R24 that they reported genes that were present in at least two databases, a sort of a voting system (Figure 7).

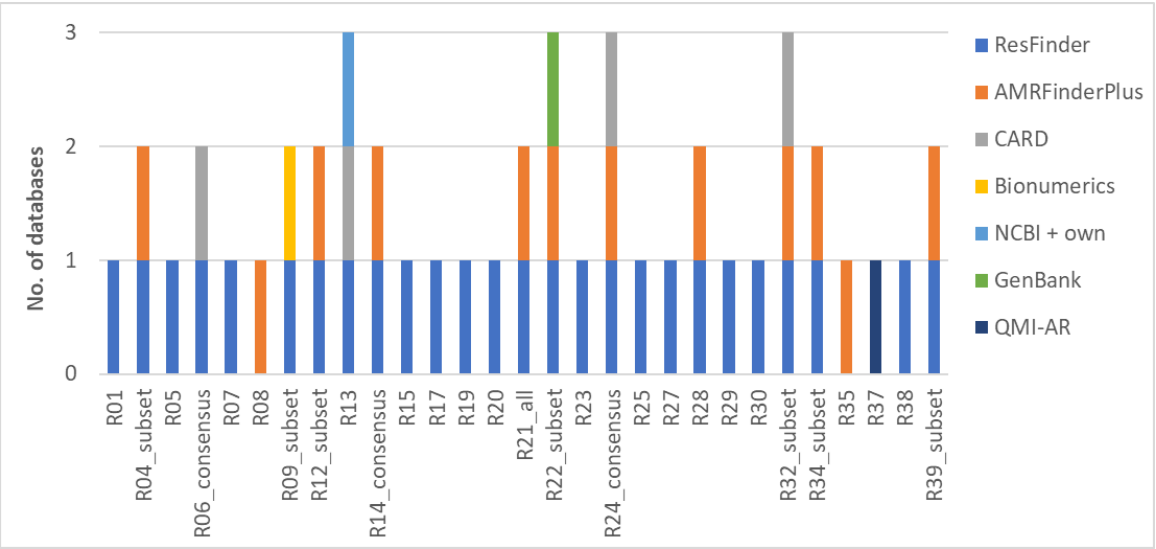

Figure 7. An overview of databases used by 29 participants for AMR gene detection in Campylobacter. The horizontal labels indicate the participant ID and how they reported AMR genes in cases when more than one database was used : consensus – common genes present in all databases used, subset – based on experience/knowledge/literature, all – genes from all databases. other – other strategy.

4.1.2. Tools and databases used for point mutations detection

All 29 participants reported the tools, the databases, and the inputs that they used for point mutations detection and also reported what approach was applied in cases when

more than one database was used. Overall, 16 unique combinations of tools/databases/inputs/reporting strategies were used by the 29 participants (Table S 4).

PointFinder was the preferred tool, being used by 24 participants, either alone or in combination with another tool. AMRFinderPlus was the second most common tool and was used by 12 participants (Figure 8).

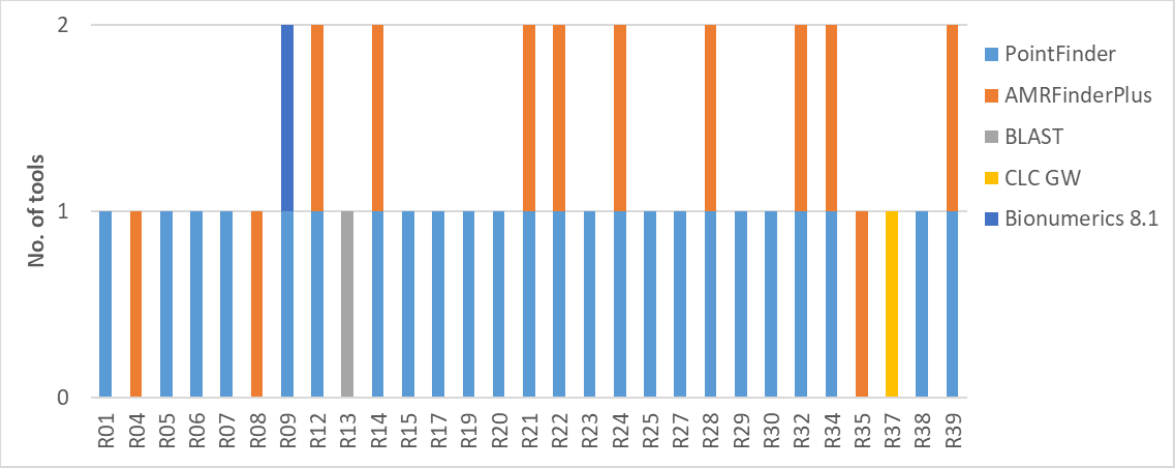

Figure 8. An overview of tools used by 29 participants for point mutations detection in Campylobacter

The majority of participants (18) used only one database (Figure 9). Among ten participants that used two databases, nine used ResFinder and AMRFinderPlus and one used a combination of ResFinder and BioNumerics. The participants that used more than one database also indicated how they had reported the point mutations (Figure 9).

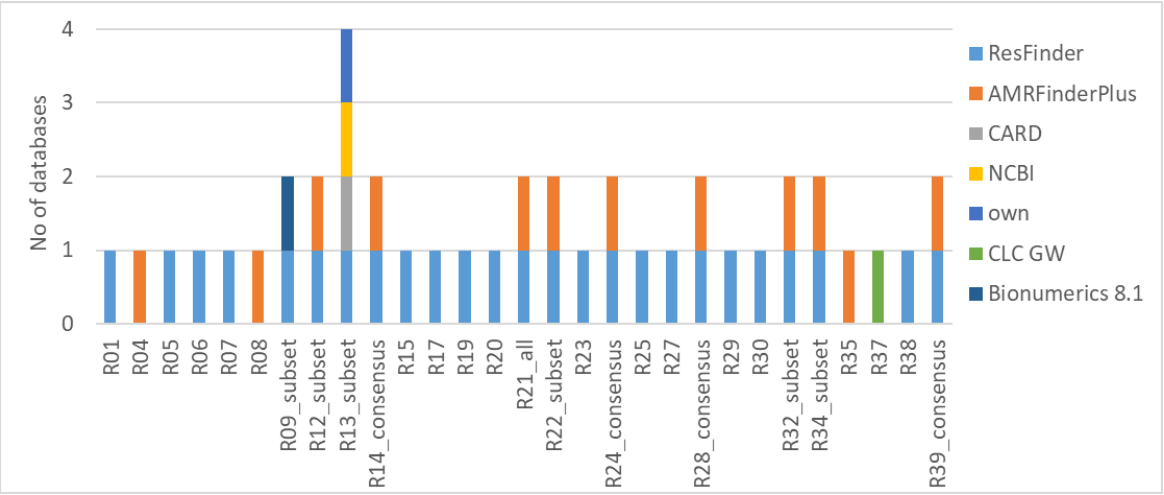

Figure 9. An overview of databases used by 29 participants for point mutations detection in Campylobacter. The horizontal labels indicate the participant ID and how they reported point mutations in cases when more than one database was used : all - reported all point mutations from all databases, a subset – reported a subset of point mutations based on experience/knowledge/literature; consensus - a consensus list of point mutations (the same mutations present in all databases used).

4.2. Species and STs reported

4.2.1. Methods used for species identification and results

All 29 participants reported the methods for *Campylobacter* species identification. Twenty-five participants applied only one method, and four participants applied two. Most commonly used methods were KmerFinder (n=14) and Kraken (n=5). Other methods were applied by 1-3 participants and are shown in Table S 7 along with the reported species.

Sequences from strains TRING2C-4, TRING2C-7 and TRING2C-9 were of *C. jejuni* species and all participants reported that correctly. The other two sequences were from *C. coli* and three participants reported that incorrectly as *C. jejuni* for sequence TRING2C-1. Two participants made a similar mistake for sequence TRING2C-10.

4.2.2. Methods used for ST identification and results

All 29 participants reported the methods used for *Campylobacter* ST identification. Each participant applied only one method. MLST2.0 (CGE tools) was the preferred method, used by fourteen participants. The other methods used are shown in Table S 8, along with the reported ST.

All but one of the participants reported correct STs for all three sequences. One laboratory reported ST830 for sequence TRING2C-10 instead of 12073. Additionally, one laboratory did not report STs for all three sequences and three laboratories did not report ST for some of the sequences (Table S 8).

4.3. AMR genes and PMs reported for *Campylobacter* strains

4.3.1. Strain TRING2C-1

Differences observed between the two reference datasets, Res_Ref and AMR_Ref, are summarised in Table 17, along with suggested explanations for the discrepancy.

Table 17. Suggested explanation of differences between reference datasets Res_Ref and AMR_Ref from Table 18, observed in gene reporting from Campylobacter strain TRING2C-1. Grey shading indicates in which database the gene was present.

Gene	Res_Ref	AMR_Ref	Suggested explanation	Reference/comment
aad9			*aad9* not present in ResFinder	
aadE			Variant in AMRFinderPlus, synonym of *ant(6)-Ia*	(9)
ant(6)-Ia			Variant in ResFinder, synonym of *aadE*	(9)
tet(O)			This gene was identified with 65% coverage and 91% identity in the AMR_Ref dataset.	The gene in this sequence is on a partial contig, most likely split during assembly, making precise identification difficult.
tet(O/32/O)			This gene was identified with 65% coverage and 99.9% identity in the Res_Ref dataset.	

Three out of six AMR genes in this sequence were reported by more than 90% of participants (Table 18). The precise variant of the gene responsible for tetracycline resistance was difficult to identify due to the likely split during assembly. Hence, the varying reporting of the gene among participants as *tet(O)* and *tet(O/32/O)*.

Table 18. AMR Genes reported in Campylobacter strain TRING2C-1. Reference datasets, Res_Ref and AMR_Ref, are shaded grey. Participants are grouped based on database(s) used : Green – ResFinder, Blue – AMRFinderPlus with or without ResFinder, Yellow – CARD with or without any other database. Percentage concordance is based on following scale : darkest orange colour : 100% concordance among participants, lighter orange colour : 90-99% concordance, lightest orange colour : 80-89% concordance. Concordance lower than 80% is without colour.

	ResFinder															AMRFinderPlus +/- ResFinder										CARD +/- others							
	Res_Ref	R19	R15	R23	R25	R29	R17	R30	R27	R07	R05	R20	R01	R38	AMR_Ref	R14	R08	R35	R12	R04	R28	R21	R34	R39	R22	R13	R06	R24	R32	R09	R37	% concordance
ResFinder																																
AMRFinderPlus																																
CARD																																
Other database																									A	B				C	D	
aad9															X	X	X	X	X	X	X	X		X	X	X			X			38
aadE									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		93
aadE-Cc	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X	X		90
blaOXA-193	X	X	X	X		X	X	X	X	X		X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		86
lnu(C)	X		X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	93
tet(O)															X												X				X	
tet(O/32/O)	X	X	X	X	X	X	X	X	X			X	X	X					X		X	X	X	X	X	X		X	X	X		79

[A] GenBank
[B] NCBI + home-made database
[C] BioNumerics 8.1
[D] QMI-AR Peptide Marker Database (2021-08)

Almost 90% participants reported the mutation in *gyrA* (Table 19). Two participants out of 14, using PointFinder, specified in comments that no point mutations were found in this sequence and the third participant, who did not report any mutations, did not elaborate in comments.

Table 19. Point mutations (PMs) reported in Campylobacter strain TRING2C-1. Reference datasets, Res_Ref and AMR_Ref, are shaded grey. Participants are grouped based on database(s) used : Green – ResFinder, Blue – AMRFinderPlus with or without ResFinder. Yellow – other database(s) (specified below the table). Percentage concordance is based on following scale : darkest orange colour : 100% concordance among participants, lighter orange colour : 90-99% concordance, lightest orange colour : 80-89% concordance. Concordance lower than 80% is without colour.

	PointFinder															AMRFinderPlus +/- PointFinder													Other		
	Res_Ref	R37	R15	R19	R23	R25	R17	R30	R27	R07	R05	R20	R01	R06	R38	AMR_Ref	R08	R04	R35	R14	R12	R24	R32	R28	R21	R22	R34	R39	R13	R09	% concordance
PointFinder																															
AMRFinderPlus																															
Other database																													*	**	
gyrA_2				X						X																					
gyrA T86I						X			X							X	X	X	X	X			X	X	X			X	X		89
gyrA_2 T86I	X		X				X	X				X	X	X	X						X	X				X	X			X	

* Card, Ncbi, home-made database
** BioNumerics 8.1

4.3.2. Strain TRING2C-4

In sequence from strain TRING2C-4, only a beta-lactamase gene *bla*$_{OXA-461}$ was detected in both reference datasets and reported by 90% of participants (Table 20).

Table 20. AMR Genes reported in Campylobacter strain TRING2C-4. Reference datasets, Res_Ref and AMR_Ref, are shaded grey. Participants are grouped based on database(s) used : Green – ResFinder, Blue – AMRFinderPlus with or without ResFinder, Yellow – CARD with or without any other database. Percentage concordance is based on following scale : darkest orange colour : 100% concordance among participants, lighter orange colour : 90-99% concordance, lightest orange colour : 80-89% concordance. Concordance lower than 80% is without colour.

	ResFinder														AMRFinderPlus +/- ResFinder										CARD +/- others							
	Res_Ref	R19	R15	R23	R25	R29	R17	R30	R27	R07	R05	R20	R01	R38	AMR_Ref	R14	R08	R35	R12	R04	R28	R21	R34	R39	R22	R13	R06	R24	R32	R09	R37	% concordance
ResFinder																																
AMRFinderPlus																																
CARD																																
Other database																									A	B				C	D	
blaOXA-461	X	X	X	X	X		X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		90

[A] GenBank
[B] NCBI + home-made database
[C] BioNumerics 8.1
[D] QMI-AR Peptide Marker Database (2021-08)

4.3.3. Strain TRING2C-7

Differences observed between the two reference datasets, Res_Ref and AMR_Ref, are summarised in Table 21, along with suggested explanations for the discrepancy.

Table 21. Suggested explanation of differences between reference datasets Res_Ref and AMR_Ref from Table 22, observed in gene reporting from Campylobacter strain TRING2C-7. Grey shading indicates in which database the gene was present.

Gene	Res_Ref	AMR_Ref	Suggested explanation	Reference/comment
aad9			*aad9* not present in ResFinder	
aph(3')-III			High similarity (99.5%) with *aph(3')-IIIa* variant that also is present in ResFinder database.	
aph(3')-IIIa			This is one of many similar variants of aph(3')-III gene present in AMRFinderPlus database. This one had a match of 100% identity and 100% coverage.	
cat			More variants of the cat gene are present in ResFinder database, but this one was identified with 100% coverage and identity.	
catA13			Variant present in AMRFinderPlus database and identified with 100% coverage and identity.	
tet(O)			Gene present in ResFinder. It was not detected in ResFinder when reads were used (in April 2023), but detected when SPAdes assemblies were used. Since the provider reported results obtained only with reads in Res_Ref dataset, this gene was not reported in May.	Of note, when provider submitted reads as input to ResFinder in November 2023 again, the *tet(O)* gene was detected also when reads were used as input.

High concordance for three genes was observed in sequence TRING2C-7, when taking into consideration that the same gene was reported in various forms for two of the genes: *catA13* and *aph(3')-IIIa* (Table 22).

The reasons for only one out of nine participants from the blue group (AMRFinderPlus) reporting the gene *aad9* are unclear. In the AMR_Ref dataset, the gene was detected with 84% coverage and 100% identity. For this reason, this gene could have been potentially missed by four participants from the blue group: R04, R08, R21 and R35 who applied identity thresholds higher or equal to 90%. However, the other three participants used identity thresholds as low as 50% and 60%, so the gene should have been

detected. The one participant from the blue group that did report this gene, used the default thresholds for AMRFinderPlus (90% identity and 50% coverage (4)).

The provider's issues encountered with the gene *tet(O)* are described in Table 21. It is unknown whether the six participants in the green group (ResFinder), who did not report the gene, also encountered a similar problem. All six participants used reads as input.

Table 22. AMR Genes reported in Campylobacter strain TRING2C-7. Reference datasets, Res_Ref and AMR_Ref, are shaded grey. Participants are grouped based on database(s) used : Green – ResFinder, Blue – AMRFinderPlus with or without ResFinder, Yellow – CARD with or without any other database. Percentage concordance is based on following scale : darkest orange colour : 100% concordance among participants, lighter orange colour : 90-99% concordance, lightest orange colour : 80-89% concordance. Concordance lower than 80% is without colour.

	ResFinder															AMRFinderPlus +/- ResFinder										CARD +/- others							
	Res_Ref	R19	R15	R23	R25	R29	R17	R30	R27	R07	R05	R20	R01	R38	AMR_Ref	R14	R08	R35	R12	R04	R28	R21	R34	R39	R22	R13	R06	R24	R32	R09	R37	% concordance
ResFinder																																	
AMRFinderPlus																																	
CARD																																	
Other database																									A	B				C	D		
aad9															X						X				X	X						10
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	97
aph(3')						X																											
aph(3')-III	X		X		X		X	X	X	X		X							X			X	X										
aph(3')-IIIa		X									X		X	X	X	X	X			X	X	X		X	X	X	X	X	X	X	X	97
blaOXA-193	X	X	X	X		X	X	X	X			X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		86
cat	X	X	X	X	X	X	X	X	X	X	X	X	X	X								X				X		X		X	X	
catA13															X	X	X	X	X	X	X	X	X	X	X			X	X			97
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		
tet(O/32/O)				X																												83

[A] GenBank
[B] NCBI + home-made database
[C] BioNumerics 8.1
[D] QMI-AR Peptide Marker Database (2021-08)

All participants reported the T86I substitution in gene *gyrA* in sequence TRING2C-7, whereas 34% of participants reported the mutation *50S* L22 A103V, only present in the AMRFinderPlus database. The correlation between this mutation and macrolide resistance has not been confirmed (10, 11).

Table 23. Point mutations (PMs) reported in Campylobacter strain TRING2C-7. Reference datasets, Res_Ref and AMR_Ref, are shaded grey. Participants are grouped based on database(s) used : Green – ResFinder, Blue – AMRFinderPlus with or without ResFinder. Yellow – other database(s) (specified below the table). Percentage concordance is based on following scale : darkest orange colour : 100% concordance among participants, lighter orange colour : 90-99% concordance, lightest orange colour : 80-89% concordance. Concordance lower than 80% is without colour.

	PointFinder																AMRFinderPlus +/- PointFinder														Other		
	Res_Ref	R37	R15	R19	R23	R25	R29	R17	R30	R27	R07	R05	R20	R01	R06	R38	AMR_Ref	R08	R04	R35	R14	R12	R24	R32	R28	R21	R22	R34	R39	R13	R09	% concordance
PointFinder																																	
AMRFinderPlus																																	
Other database																														*	**		
50S L22 A103V																	X	X	X			X	X	X	X	X	X	X	X			34
gyrA				X							X					X																	
gyrA T86I	X	X	X		X	X	X	X	X	X		X	X	X	X		X	X	X	X	X	X	X	X	X	X	X		X	X	X		
gyrA_2 p.T86I																												X				100	

* Card, Ncbi, home-made database
** BioNumerics 8.1

4.3.4. Strain TRING2C-9

Differences observed between the two reference datasets, Res_Ref and AMR_Ref, are summarised in Table 24, along with suggested explanations for the discrepancy.

Table 24. Suggested explanation of differences between reference datasets Res_Ref and AMR_Ref from Table 25, observed in gene reporting from Campylobacter strain TRING2C-9. Grey shading indicates in which database the gene was present.

Gene	Res_Ref	AMR_Ref	Suggested explanation	Reference /comment
aadE			Variant in AMRFinderPlus, synonym of *ant(6)-Ia*	(9)
ant(6)-Ia			Variant in ResFinder, synonym of *aadE*	(9)
aph(3')-III			High similarity (99.5%) with *aph(3')-IIIa* variant that also is present in ResFinder database.	
aph(3')-IIIa			This is one of many similar variants of *aph(3')-III* gene present in AMRFinderPlus database. This one had a match of 100% identity and 100% coverage.	
sat4			Gene absent from ResFinder database (no genes from streptothricin antibiotics group present).	(12)
tet(O)			Gene identified by ResFinder only when reads are used as input. The results suggest a *tet(O)* gene with identity and coverage thresholds above 90%.	
tet(O/32/O)			Gene identified by ResFinder only when reads are used as input. The results suggest a *tet(O/32/O)* gene with identity and coverage thresholds of 100%.	

Nine out of thirteen participants from the green group (ResFinder) reported the *blaOXA-193* gene, which is fewer than for the blue and yellow groups (Table 25). From the provider's examination, it is visible that analysis with ResFinder using reads as input gives the *blaOXA-193* gene as a result, whereas analysis with assemblies gives six other *blaOXA* genes in addition to *blaOXA-193* as a result. Those genes were reported by laboratories R29, R17 and R20 (Table S 17). Out of the four participants from the green group that did not report *blaOXA-193*, two used assemblies as input and two used both reads and assemblies. The *blaOXA-193* gene should have been detected in both cases, so it is unclear why it was not reported.

There was only 62% concordance in reporting the tetracycline resistance gene among the participants. Of note, none of the three participants using AMRFinderPlus alone reported the tetracycline resistance gene, which is in agreement with the AMR_Ref dataset. In the green group, seven participants that used reads as input, reported the *tet(O)* or *tet(O/32/O)* gene, in accordance with Res_Ref dataset. Out of the six participants from the green group that did not report any *tet* genes, all used assemblies as input apart from one participant that used both reads and assemblies. This is also in accordance with provider's analysis (Table 24) that shows that the *tet* gene(s) in this sequence can only be detected using ResFinder when reads are used as input (data not shown).

Table 25. AMR Genes reported in Campylobacter strain TRING2C-9. Reference datasets, Res_Ref and AMR_Ref, are shaded grey. Participants are grouped based on database(s) used : Green – ResFinder, Blue – AMRFinderPlus with or without ResFinder, Yellow – CARD with or without any other database. Percentage concordance is based on following scale : darkest orange colour : 100% concordance among participants, lighter orange colour : 90-99% concordance, lightest orange colour : 80-89% concordance. Concordance lower than 80% is without colour.

	ResFinder														AMRFinderPlus +/- ResFinder										CARD +/- others							
	Res_Ref	R19	R15	R23	R25	R29	R17	R30	R27	R07	R05	R20	R01	R38	AMR_Ref	R14	R08	R35	R12	R04	R28	R21	R34	R39	R22	R13	R06	R24	R32	R09	R37	% concordance
ResFinder																																
AMRFinderPlus																																
CARD																																
Other database																									A	B				C	D	
aadE															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		93
aph(3')						X																										
aph(3')-III	X		X		X		X	X	X	X		X							X			X	X									
aph(3')-IIIa		X	X					X	X		X		X	X	X	X	X	X		X	X	X		X	X	X	X	X	X	X	X	97
blaOXA-193	X	X	X	X		X	X	X	X			X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		83
sat4															X	X	X	X	X	X	X	X	X	X	X	X		X	X		X	48
tet(O)	X	X																					X				X					
tet(O/32/O)	X		X	X	X			X	X					X					X		X	X		X	X			X	X	X		
tet(O/M/O)																										X						62

[A] GenBank
[B] NCBI + home-made database
[C] BioNumerics 8.1
[D] QMI-AR Peptide Marker Database (2021-08)

The only point mutation present in this strain is *gyrA* T86I and it was reported by all participants as well as in both reference datasets.

Table 26. Point mutations (PMs) reported in Campylobacter strain TRING2C-9. Reference datasets, Res_Ref and AMR_Ref, are shaded grey. Participants are grouped based on database(s) used : Green – ResFinder, Blue – AMRFinderPlus with or without ResFinder. Yellow – other database(s) (specified below the table). Percentage concordance is based on following scale : darkest orange colour : 100% concordance among participants, lighter orange colour : 90-99% concordance, lightest orange colour : 80-89% concordance. Concordance lower than 80% is without colour.

	PointFinder																AMRFinderPlus +/- PointFinder													Other		
	Res_Ref	R37	R15	R19	R23	R25	R29	R17	R30	R27	R07	R05	R20	R01	R06	R38	AMR_Ref	R08	R04	R35	R14	R12	R24	R32	R28	R21	R22	R34	R39	R13	R09	% concordance
PointFinder																																
AMRFinderPlus																																
Other database																														*	**	
gyrA				X							X					X																
gyrA T86I	X	X	X		X	X	X	X	X	X		X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	100

* Card, Ncbi, home-made database
** BioNumerics 8.1

4.3.5. Strain TRING2C-10

Differences observed between the two reference datasets, Res_Ref and AMR_Ref, are summarised in Table 27 along with suggested explanations for the discrepancy.

Table 27. Suggested explanation of differences between reference datasets Res_Ref and AMR_Ref from Table 28, observed in gene reporting from Campylobacter strain TRING2C-10. Grey shading indicates in which database the gene was present.

Gene	Res_Ref	AMR_Ref	Suggested explanation	Reference /comment
aad9			*aad9* not present in ResFinder	
aadE			Variant in AMRFinderPlus, synonym of *ant(6)-Ia*	(9)
ant(6)-Ia			Variant in ResFinder, synonym of *aadE*	(9)
aph(3')-III			High similarity (99.5%) with *aph(3')-IIIa* variant that also is present in ResFinder database.	
aph(3')-IIIa			This is one of many similar variants of *aph(3')-III* gene present in AMRFinderPlus database. This one had a match of 100% identity and 100% coverage.	
catA			Gene detected with 100% identity and coverage.	
cat(pC194)			Gene detected with 100% identity and coverage. Gene variant absent from AMRFinderPlus database.	
sat4			Gene absent from ResFinder database (no genes from streptothricin antibiotics group present).	(12)
tet(O)			This gene was identified with 100% coverage and 93% identity in the AMR_Ref dataset.	
tet(O/32/O)			This gene was identified with 100% coverage and 99.8% identity in the Res_Ref dataset (regardless of whether reads or assemblies were used as input).	

All participants reported the *ermB* gene and the chloramphenicol resistance gene, albeit in four different forms: *cat*, *catA*, *cat(pC194)* or *cat-TC* (Table 28). Differences in the gene name can be attributed to which variant is present in which database. Participant R37, who reported the *cat-TC* gene, was the only one to use the QMI-AR Peptide Marker database. This database, launched by Qiagen, enables access to four large databases on antimicrobial resistance: CARD, ResFinder, AMRFinder and ARG-ANNOT database of peptide markers. It is worth noting, however, that the same participant reported the *cat-TC* gene also in sequence TRING2C-1, in which the presence of a *cat* gene was not expected (Table S 14).

Gene *aph(3')-IIIa* was reported by 55% of the participants. The gene, in both reference datasets, was reported with 93-94% identity. Only four out of thirteen participants who did not report, used thresholds that would not allow this gene to be detected, so it remains unclear why this gene was not reported by all participants.

There was 90% concordance in detecting the *tet* gene in sequence TRING2C-10 among the participants. Two out of three participants from the blue group, using only AMRFinderPlus, did not report it, in contrast to provider's AMR_Ref dataset, where the gene *tet(O)* was detected with 93% identity and 100% coverage. Since one participant, R08, applied a threshold of 97% for identity and coverage, the gene could have been missed. Participant R35, who also did not detect any *tet* genes in this strain, applied thresholds of 90% identity and 100% coverage, but reported issues with the *tet* genes in this, and other *Campylobacter* sequences. They reported that in TRING2C-10 the *tet(O/32/O)* gene was detected using ResFinder (used as a supplementary tool) but not AMRFinderPlus. It is, however, unclear why the *tet(O)* gene was not reported by this country.

Table 28. AMR Genes reported in Campylobacter strain TRING2C-10. Reference datasets, Res_Ref and AMR_Ref, are shaded grey. Participants are grouped based on database(s) used : Green – ResFinder, Blue – AMRFinderPlus with or without ResFinder, Yellow – CARD with or without any other database. Percentage concordance is based on following scale : darkest orange colour : 100% concordance among participants, lighter orange colour : 90-99% concordance, lightest orange colour : 80-89% concordance. Concordance lower than 80% is without colour.

	ResFinder														AMRFinderPlus +/- ResFinder										CARD +/- others							
	Res_Ref	R19	R15	R23	R25	R29	R17	R30	R27	R07	R05	R20	R01	R38	AMR_Ref	R14	R08	R35	R12	R04	R28	R21	R34	R39	R22	R13	R06	R24	R32	R09	R37	% concordance
ResFinder																																
AMRFinderPlus																																
CARD																																
Other database																									A	B				C	D	
aad9															X	X	X	X	X	X	X	X		X	X	X			X			38
aadE															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		97
aph(3')						X																			X							
aph(3')-III	X						X			X		X																				
aph(3')-IIIa		X		X									X		X	X	X			X	X	X		X		X		X		X		59
blaOXA-193	X	X	X	X		X	X	X	X			X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	83
cat		X				X	X	X	X	X		X	X	X												X	X	X				
catA															X	X	X	X	X	X	X	X	X	X	X				X			
cat(pC194)	X		X	X	X						X											X								X		
cat-TC																															X	100
ermB	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	100
sat4															X	X	X	X	X	X	X	X	X	X	X	X		X	X		X	48
tet(O)											X				X	X					X	X					X					
tet(O/32/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		90

[A] GenBank
[B] NCBI + home-made database
[C] BioNumerics 8.1
[D] QMI-AR Peptide Marker Database (2021-08)

Both point mutations present in sequence TRING2C-10, *gyrA* T86I and *rpsL* K43R were identified in both reference datasets, but they were reported by 90% and 86% of participants, respectively.

Table 29. Point mutations (PMs) reported in Campylobacter strain TRING2C-10. Reference datasets, Res_Ref and AMR_Ref, are shaded grey. Participants are grouped based on database(s) used : Green – ResFinder, Blue – AMRFinderPlus with or without ResFinder. Yellow – other database(s) (specified below the table). Percentage concordance is based on following scale : darkest orange colour : 100% concordance among participants, lighter orange colour : 90-99% concordance, lightest orange colour : 80-89% concordance. Concordance lower than 80% is without colour.

	PointFinder																AMRFinderPlus +/- PointFinder														Other		
	Res_Ref	R37	R15	R19	R23	R25	R29	R17	R30	R27	R07	R05	R20	R01	R06	R38	AMR_Ref	R08	R04	R35	R14	R12	R24	R32	R28	R21	R22	R34	R39	R13	R09	% concordance
PointFinder																																
AMRFinderPlus																																
Other database																														*	**	
gyrA T86I						X				X							X	X	X	X	X		X	X	X	X			X	X		
gyrA_2				X							X					X																
gyrA_2 T86I	X		X				X	X	X				X	X	X							X					X	X			X	90
rpsL				X												X																
rpsL K43R	X	X	X		X	X	X		X	X		X	X		X		X	X	X	X	X	X	X	X	X	X	X	X	X		X	86

* Card, Ncbi, home-made database
** BioNumerics 8.1

5. Conclusions

This RingTrial2-WGS-AMR (called RingTrial2), organised by Statens Serum Institut, is the second out of three exercises in the FWD AMR-RefLabCap project, spanning over 3 years. The aim of this ring trial is to investigate the outcome of different databases, tools and analytic pipelines used and enable the participants to compare their performance in AMR gene and point mutation detection.

In RingTrial2, sequences (reads and assemblies) were shared with all participants through an FTP server at the end of May 2023. Initially, the provider shared the fastq sequences in zip-compressed folders (two read files, R1 and R2, in one folder). However, in the second half of June it was reported by one of the participants that one of the sequences for TRING2S-7 was corrupted and they could not use it (contained additional characters in rows). After investigation, it was concluded that the corruption occurred during compression of the two read files, however, it did not concern the fasta files that were shared separately and without compression. The provider then replaced the compressed folders with single files on the FTP server, notified all participants of the issue via email and reopened all submission forms, in case participants wished to double check their results for this sequence. No other participant reported issues with this sequence. Thirty-five participants submitted results for both pathogens.

Many discrepancies observed in gene and point mutation reporting among the participants in this ring trial can be attributed to presence or absence of different variants in different databases or different nomenclature used. Being aware of those differences, for example gene synonyms such as *aadE* and *ant(6)-Ia* in *Campylobacter*, it is possible to compare results obtained from different databases.

Differences in gene reporting were also observed depending on whether reads or assemblies were used as input. This was noted for gene *arr-3* in TRING2S-1 for *Salmonella* and for genes *tet(O)* and *tet(O/32/O)* in *Campylobacter*, but, interestingly, only in sequence TRING2C-9, even though participants reported those genes in 4 out of 5 *Campylobacter* sequences.

Certain challenges were observed by the provider and noted by some participants when detecting tetracycline resistance genes in *Campylobacter* sequences in general. Whenever a *tet* gene was detected, it was reported as *tet(O)* by the AMRFinderPlus database and as *tet(O/32/O)* by ResFinder database, however, with certain challenges or exceptions. For example, in sequence TRING2C-7, no *tet* genes were detected in ResFinder (by the provider) when reads were used for analysis performed in April 2023. However, in the same analysis performed in November 2023, the gene was detected in reads. In TRING2C-1, according to the provider's AMRFinderPlus analysis, the *tet(O)* gene was detected, but likely split during an assembly. In sequence TRING2C-9, no *tet* genes were identified when AMRFinderPlus database was used. In the same sequence, two *tet* genes were identified in ResFinder, but only when reads were used as input, suggesting a possible issue with assembly of this gene. Similar issues were described before by Dahl et al (10) where detection of *tet(O)* or *tet(O/32/O)* gene was dependent on the methods applied. The *tet(O/32/O)* gene was identified in 27 strains by a mapping-based modified KMA approach, but not by any of the assembly-based methods used (ARIBA or ResFinder Batch Upload). This shows that tetracycline resistance based on genotypic data can be potentially missed in *Campylobacter* when only assembly-based methods are used.

Discrepancies in some results in this ring trial originated from quite high identity and coverage thresholds applied by some participants. One example of that could be the lack of reporting of gene *aad9* in sequence TRING2C-7 by four participants that used higher cut-offs for coverage than 84% which was the coverage for this gene. At the same time, several other participants did not report this gene, in spite of using cut-offs that would allow them to identify this gene.

The phenotypic testing results made available by the RingTrial provider (Table 1 and Table 2) will enable the participants to correlate their genotypic results (predictions) with the provided phenotypes for the tested antimicrobials. However, due to limitations in the number of antimicrobials tested, this correlation cannot be established for all detected genes. For example, the RingTrial provider could not confirm the phenotype-genotype correlation between the gene *lnuC* in strain TRING2C-1 and its phenotype as lincosamide susceptibility testing was not performed. The issue is similar for the *lnuF* gene in *Salmonella* strain TRING2S-1.

Likewise, the presence of *blaOXA* genes in all *Campylobacter* strains cannot be attributed to a beta-lactam resistant phenotype in all the strains, as only Ertapenem was tested from this group of antimicrobials and that resulted in resistant phenotype only in two

out of five strains tested. In general, correlating the presence of *blaOXA* genes in *Campylobacter* to phenotypic resistance is complicated. It was shown that the presence of a G to T mutation in the promoter of the *blaOXA-61* gene was the reason for resistance to ampicillin and not the presence of the gene alone (13, 14). Molecular basis for resistance to carbapenems, such as Ertapenem, was recently demonstrated to be due to insertion of an extra Aspartic acid (D) in the major outer protein PorA, as well as a point mutation elsewhere in the protein (15). This new mechanism is not yet included in antimicrobial databases.

Participants in RingTrial2 used 25 and 24 unique combinations of tools, databases, inputs and thresholds for gene detection in *Salmonella* and *Campylobacter*, respectively (Table S 1 and Table S 3). For point mutation detection, 15 and 16 unique combinations were applied for *Salmonella* and *Campylobacter*, respectively (Table S 2 and Table S 4).

Despite the differences in reporting of certain genes, the results in this RingTrial2 were comparable among participants using a variety of approaches. For many sequences, more than 90% of participants reported the same gene. The provider will use the experiences collected in this round to improve the final round of the RingTrial, for example by including the possibility to report the predicted phenotype for those antimicrobials that were phenotypically tested by the RingTrial provider.

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7. Annex A

7.1. Supplementary materials, methods for gene and point mutation detection

Table S 1. An overview of the tools, databases, inputs, thresholds for sequence coverage and identity used by 29 participants for the detection and reporting of AMR genes in Salmonella. Same number in the first column indicates that these participants used same tools and databases with the same inputs, identity, coverage and same strategy of reporting.

	Tools/ Inputs[A]	Databases/ Inputs[A]	No. of participants	Participant ID[B]	Identity (%)	Coverage (%)
	1 tool, 1 input	1 database, 1 input				
1	ResFinder_N	ResFinder_N	2	R18	30	20
2				R20	90	60
3	ResFinder_R	ResFinder_R	6	R15	90	60
3				R17	90	60
3				R30	90	60
3				R33	90	60
4				R23	85	60
5				R38	80	60
6	AMRFinderPlus_N	AMRFinderPlus_N	2	R01	default[C]	default[C]
7				R35	97	97
8	AMRFinderPlus_R	AMRFinderPlus_P	1	R08	>90	100
9	AbriTAMR 1.0.13	AbriTAMR 1.0.13	1	R10	default	default
	1 tool, >1 input	1 database, >1 input				
10	ResFinder_N_R	ResFinder_N_R	3	R25	99	100
11				R27	90	60
11				R31	90	60
12	ResFinder_N_P_R	ResFinder_N_P_R	1	R07	90	60
	1 tool, 1 input	2 databases, 1 input				
13	Bionumerics	Bionumerics/ ResFinder[D]	1	R40	>98	100
	1 tool, >1 input	2 databases, >1 input				
14	ResFinder_N_R	ResFinder_N_R/ CARD_N	1	R06[E]	90	60
	2 tools, 1 input	1 database, 1 input				
15	ResFinder_N/ ABRicate_N	ResFinder_N	1	R05	90	60
	2 tools, 1 input	2 databases, 1 input				
16	ResFinder_N/ AMRFinderPlus_N	ResFinder_N/ AMRFinderPlus_N	1	R14	90	60
17	ResFinder_R/ AMRFinderPlus_R	ResFinder_R/ AMRFinderPlus_R	1	R21	>90	
	2 tools, >1 input	2 databases, >1 input				
18	ResFinder_R/ AMRFinderPlus_N	ResFinder_R/ AMRFinderPlus_N	2	R28[F]	default	default
19				R34	90	60
20	ResFinder_N_R/ AMRFinderPlus_N	ResFinder_N_R/ AMRFinderPlus_N	1	R39	90	50
	Tools/ Inputs[A]	**Databases/ Inputs[A]**	**No. of participants**	**Participant ID[B]**	**Identity (%)**	**Coverage (%)**
	3 tools, >1 input	2 databases, >1 input				

21	AMRFinderPlus_N[G]/ ARIBA_R/ABRicate_R	AMRFinderPlus_N/ ResFinder_R	1	R04	90/85/90[H]	90/85/90[H]
22	ResFinder_N_R/ AMRFinderPlus_N/ BLAST_N	ResFinder_N_R/ AMRFinderPlus_N	1	R22	90	40
	3 tools, >1 input	3 databases, >1 input				
23	ResFinder_N_R/ AMRFinderPlus_N/ RGI_N	ResFinder_N_R/ AMRFinderPlus_N/ CARD_N	2	R02	90	60
24				R24[I]	default	90
25	ResFinder_R/ AMRFinderPlus_N/ RGI_N	ResFinder_R/ AMRFinderPlus_N/ CARD_N	1	R32	at least 99[J]	at least 99[J]

[A]Inputs: N - DNA fasta, P - protein fasta, R - raw reads. >1 input, if different inputs were used for at least one of the tools/databases
[B]In the partcipant ID column: light yellow indicated that theses participants reported reporting all genes from all databases, light red - that participants reported a subset of genes based on experience/knowledge/literature, light green - that participants reported a consensus list of genes (common genes present in all databases used).
[C]present in Ridom SeqSphere
[D]ResFinder for 1 Salmonella strain
[E]In my report I have also used the literature where I found it necessary
[F]I am reporting consensus and subset of genes that were only in one of databases
[G]Bionumerics Plugin (AMRFinder)
[H]AMRFinder (90); BN plugin (85), ResFinder (90)
[I]we report genes that are present in at least 2 databases, a sort of a voting system
[J]on one of the three databases used

Table S 2. An overview of tools, databases and inputs used by 29 participants for the detection and reporting of point mutations in Salmonella. Same number in the first column indicates that these participants used same tools and databases with the same inputs, and same strategy of reporting.

	Tools/Inputs[A]	Databases/Inputs[A]	No. of participants	Participant ID[B]
	1 tool,/1 input	1 database/ 1 input		
1	PointFinder_N	ResFinder_N	4	R18
1				R20
1				R06
1				R25
2	PointFinder_R	ResFinder_R	6	R15
2				R17
2				R23
2				R30
2				R33
2				R38
3	AMRFinderPlus_N	AMRFinderPlus_N	2	R01
3				R35
4	AMRFinderPlus_N[C]	AMRFinderPlus_N	1	R04
5	AMRFinderPlus_R[D]	AMRFinderPlus_P	1	R08
6	AbriTAMR 1.0.13	AbriTAMR 1.0.13	1	R10
7	Bionumerics	Bionumerics	1	R40
	1 tool/>1 input	1 database/>1 input		
8	PointFinder_N_R	ResFinder_N_R	2	R27
8				R31
9	PointFinder_N_P_R	ResFinder_N_P_R	1	R07
	2 tools/1 input	2 databases/1 input		
10	PointFinder_N/ AMRFinderPlus_N	ResFinder_N/ AMRFinderPlus_N	2	R14
10				R22
11	PointFinder_R/ AMRFinderPlus_R	ResFinder_R/ AMRFinderPlus_R	1	R21
	2 tools/>1 input	2 databases/>1 input		
12	PointFinder_R/ AMRFinderPlus_N	ResFinder_R/ AMRFinderPlus_N	3	R28
12				R32
12				R34
13	PointFinder_N_R/ AMRFinderPlus_N	ResFinder_N_R/ AMRFinderPlus_N	3	R02
14				R24
14				R39
	3 tools/1 input	1 database/1 input		
15	PointFinder_N/ ABRicate_N/StarAMR	ResFinder_N	1	R05

[A]Inputs: N - DNA fasta, P - protein fasta, R - raw reads. >1 input, if different inputs were used for at least one of the tools/databases
[B]In the partcipant ID column: light yellow indicated that theses participants reported point mutations from all databases without curating, light red - that these participants curated the point mutations from all databases for duplicates
[C]Bionumerics Plugin (AMRFinder)
[D]AMRFInderPlus in Ridom SeqSphere

Table S 3. An overview of the tools, databases, inputs, thresholds for sequence coverage and identity used by 29 participants for the detection and reporting of AMR genes in Campylobacter. Same number in the first column indicates that these participants used same tools and databases with the same inputs, identity, coverage and same strategy of reporting.

	Tools/ Inputs[A]	**Databases/ Inputs[A]**	**No. of participants**	**Participant ID[B]**	**Identity (%)**	**Coverage (%)**
	1 tool/1 input	1 database/1 input				
1	ResFinder_N	ResFinder_N	5	R01	90	60
1				R05	90	60
1				R17	90	60
1				R20	90	60
2				R29		
3	ResFinder_R	ResFinder_R	5	R15	90	60
3				R19	90	60
4				R23	85	60
3				R30	90	60
5				R38	80	60
6	AMRFinderPlus_N	AMRFinderPlus_N	1	R35	97	97
7	AMRFinderPlus_R	AMRFinderPlus_P	1	R08	>90	100
8	CLC GW	QMI-AR	1	R37	98	60
	1 tool/1 input	4 databases/1 input				
9	BLAST_N	ResFinder_N/ CARD_N/NCBI/ own	1	R13	90	90
	1 tool/>1 input	1 database/>1 input				
10	ResFinder_N_R	ResFinder_N_R	2	R25	99	100
11				R27	90	60
12	ResFinder_N_P_R	ResFinder_N_P_R	1	R07	90	60
	2 tools/1 input	2 databases/1 input				
13	ResFinder_N/ AMRFinderPlus_N	ResFinder_N/ AMRFinderPlus_N	1	R14	90	60
14	ResFinder_R/ AMRFinderPlus_R	ResFinder_R/ AMRFinderPlus_R	1	R21	>90	
15	ResFinder_N/ Bionumerics v8.1	ResFinder_N/ Bionumerics v8.1	1	R09	90	60
	2 tools/>1 input	2 databases/>1 input				
16	ResFinder_N_R/ AMRFinderPlus_N	ResFinder_N_R/ AMRFinderPlus_N	1	R12	90	60
17	ResFinder_R/ AMRFinderPlus_N	ResFinder_R/ AMRFinderPlus_N	2	R28[C]	default	default
18				R34	90	60
19	ResFinder_N_R/ RGI_N	ResFinder_N_R/ CARD_N	1	R06[D]	90	60
	3 tools/>1 input	2 databases/>1 input				
20	AMRFinderPlus_N/ ARIBA_R/ ABRicate_R	AMRFinderPlus_N/ ResFinder_R	1	R04	90	90
	Tools/ Inputs[A]	**Databases/ Inputs[A]**	**No. of participants**	**Participant ID[B]**	**Identity (%)**	**Coverage (%)**
	3 tools/>1 input	3 databases/1 input				
21	ResFinder_N_R/ AMRFinderPlus_N/ BLAST_N	ResFinder_N/ AMRFinderPlus_N/ GenBank	1	R22	90	40
22	ResFinder_N_R/ AMRFinderPlus_N/ RGI_N	ResFinder_N_R/ AMRFinderPlus_N/ CARD_N	1	R24[E]	default	90

23	ResFinder_N_R/ AMRFinderPlus_N/ startamr	ResFinder_N_R/ AMRFinderPlus_N	1	R39	90	50
24	ResFinder_R/ AMRFinderPlus_N/ RGI_N	ResFinder_R/ AMRFinderPlus_N/ CARD_N	1	R32	at least 99[F]	at least 99[F]

[A]Inputs: N - DNA fasta, P - protein fasta, R - raw reads. >1 input, if different inputs were used for at least one of the tools/databases
[B]In the partcipant ID column: light yellow indicated that theses participants reported reporting all genes from all databases, light red - that participants reported a subset of genes based on experience/knowledge/literature, light green - that participants reported a consensus list of genes (common genes present in all databases used).
[C]consensus and subset of genes found by one of databases
[D]also used the literature where necessary
[E]we report genes that are present in at least 2 databases, a sort of a voting system
[F]at least 99% on one of the three databases used

Table S 4. An overview of tools, databases and inputs used by 29 participants for the detection and reporting of point mutations in Campylobacter. Same number in the first column indicates that these participants used same tools and databases with the same inputs, and same strategy of reporting.

	Tools/Inputs[A]	Databases/Inputs[A]	No. of participants	Participant ID[B]
	1 tool/1 input	1 database/1 input		
1	ResFinder _N	ResFinder_N	7	R01
1				R05
1				R06
1				R17
1				R20
1				R25
1				R29
2	ResFinder _R	ResFinder _R	5	R15
2				R19
2				R23
2				R30
2				R38
3	AMRFinderPlus_N	AMRFinderPlus_N	2	R04
3				R35
4	AMRFinderPlus_R	AMRFinderPlus_R	1	R08
5	CLC GW[C]	CLC GW[C]	1	R37
	1 tool/>1 input	1 database/>1 input		
6	ResFinder _N_R	ResFinder _N_R	1	R27
7	ResFinder _N_P_R	ResFinder _N_P_R	1	R07
	1 tool/1 input	4 databases/1 input		
8	BLAST_N	ResFinder _N/ CARD/NCBI/own	1	R13
	2 tools/1 input	2 databases/1 input		
9	ResFinder _N/ AMRFinderPlus_N	ResFinder _N/ AMRFinderPlus_N	2	R14
10				R22
11	ResFinder _R/ AMRFinderPlus_R	ResFinder _R/ AMRFinderPlus_R	1	R21
12	ResFinder _N/ Bionumerics 8.1	ResFinder _N/ Bionumerics 8.1	1	R09
	2 tools/>1 input	2 databases/>1 input		
13	ResFinder _N_R/ AMRFinderPlus_N	ResFinder _N_R/ AMRFinderPlus_N	3	R12
14				R24
14				R39
15	ResFinder _R/ AMRFinderPlus_N	ResFinder _R/ AMRFinderPlus_N	3	R28
16				R32
16				R34

[A]Inputs: N - DNA fasta, P - protein fasta, R - raw reads. >1 input, if different inputs were used for at least one of the tools/databases
[B]In the participant ID column: light yellow indicated that theses participants reported point mutations from all databases, light red - a subset of point mutations based on experience/knowledge/literature; light green - a consensus list of point mutations (the same mutations present in all databases used)
[C]CLC Genomics Workbench Microbial genomic module PointFinder database for Campylobacter (2019-08)

7.2. Supplementary materials, serotype/species and ST identification

Table S 5. Salmonella serotypes identified by the participants

Lab code	TRING2S-1	TRING2S-2	TRING2S-4	TRING2S-7	TRING2S-10
R01	S. Kentucky	S. Typhimurium	S. Poona	1,4,5,12:I:-	1,4,(5):12:I:-
R02	Saintpaul	Meleagridis	Typhimurium	Newport	1,4,[5],12:i:- (Typhimurium - monophasic)
R04	Saintpaul	Meleagridis	Typhimurium	Newport	Monophasic Typhimurium
R05	Saintpaul	Meleagridis	Typhimurium	Newport	Typhimurium
R06	Saintpaul (4:e,h:1,2)	S. Meleagridis (3,10:e,h:l,w)	S. Typhimurium (4:i:1,2)	S. Newport (8:e,h:1,2)	Monophasic variant of S. Typhimurium (4:i:-)
R07	O -4 : H1 e,h : H2 1,2	O -3,10 : H1 e,h : H2 1,w	O -4 : H1 i : H2 1,2	O -8 : H1 e,h : H2 1,2	O -4, H1 i, H2 ---
R08	Saintpaul	Meleagridis	Typhimurium	Newport	monophasic Salmonella Typhimurium (I 4,[5],12:i:-)
R10	Saintpaul	Meleagridis	Typhimurium	Newport	I 4,[5],12:i:-
R14	4:e,h:1,2	3,10:e,h:l,w	4:i:1,2	8:e,h:1,2	4:i:-
R15	4:e,h:1,2 Saintpaul	3,10:e,h:l,w Meleagridis	4:i:1,2 Typhimurium	8:e,h:1,2 Newport*	4:i:- O5- variant of Typhimurium
R17	Saintpaul	Meleagridis	Typhimurium	Newport	potential monophasic variant of Typhimurium
R18	Saintpaul	Meleagridis	Typhimurium	Newport	I. 4,12:i:- (monophasic)
R20	Salmonella Saintpaul 4:e,h:1,2	Salmonella Meleagridis 3,10:e,h:l,w	Salmonella Typhimurium 4:i:1,2	Salmonella Newport 8:e,h:1,2	Salmonella potential monophasic variant of Typhimurium 4,[5],12:i:-
R21	Saintpaul	Meleagridis	Typhimurium	Newport	monophasic variant of Typhimurium; 1,4,[5],12:i:-
R22	Saintpaul	Meleagridis	Typhimurium	Newport	monophasic variant of Typhimurium
R23	Saintpaul	Meleagridis	Typhimurium	Newport	Typhimurium (monophasic)
R24	Saintpaul	Meleagridis	Typhimurium	Newport	Monophasic Typhimurium
R25	Saintpaul	Meleagridis			potential monophasic variant of Typhimurium(O5-)
R27	Saintpaul	Meleagridis	Typhimurium	Newport	monophasic Typhimurium
R28	Saintpaul	Meleagridis	Typhimurium	Newport	I 4,[5],12:i:-
R30	Salmonella Saintpaul	Salmonella Meleagridis	Salmonella Typhimurium	Salmonella Newport	Monophasic Salmonella Typhimurium
R31	Salmonella Saintpaul	Salmonella Meleagridis	Salmonella Typhimurium (O5-)	Salmonella Newport	Salmonella Tiphymurium monophasic variant (O5-)
R32	Saintpaul	Meleagridis	Typhimurium	Newport	Typhimurium
R33	monophasic Typhimurium (4:i:-)	Meleagridis (3,10:e,h:l,w)	Typhimurium (4:i:1,2)	Newport (8:e,h:1,2)	monophasic Typhimurium (4:i:-)
R34	Saintpaul 4:e,h:1,2	Meleagridis 3,10:e,h:l,w	Typhimurium 4:i:1,2	Newport 8:e,h:1,2	Monophasic Typhimurium 4:i:-
R35	Saintpaul	Meleagridis	Typhimurium	Newport	monophasic Typhimurium
R38	Saintpaul (4:e,h:1,2)	Meleagridis (3,10:e,h:l,w)	Typhimurium (4:i:1,2)	Newport (8:e,h:1,2)	potential monophasic variant of Typhimurium (4:i)

R39	Saintpaul	Meleagridis	Typhimurium	Newport	Typhimurium monophasic variant
R40	Saintpaul	Meleagridis	Typhimurium	Newport	I 4,[5],12:i:-

Table S 6. Salmonella ST and methods used for identification by the participants

Lab code	**method**	**TRING2S-1**	**TRING2S-2**	**TRING2S-4**	**TRING2S-7**	**TRING2S-10**
R01	Risom SeqSphere	50	463	19	132	34
R02	MLST2.0 (CGE tools)	50	463	19	132	34
R04	In-house Bifrost (read mapping) to Enterobase scheme	50	463	19	132	34
R05	MLST2.0 (CGE tools)	50	463	19	132	34
R06	MLST2.0 (CGE tools)	50	463	19	132	34
R07	MLST2.0 (CGE tools)	50	463	19	132	34
R08	Enterobase scheme run in SeqSphere+	50	463	19	132	34
R10	Enterobase	50	463	19	132	34
R14	MLST (tsemann)	50	463	19	132	34
R15	MLST2.0 (CGE tools)	50	463	19	132	34
R17	MLST2.0 (CGE tools)	50	463	19	132	34
R18	Enterobase	50	463	19	132	34
R20	MLST2.0 (CGE tools)	50	463	19	132	34
R21	MLST2.0 (CGE tools)	50	463	19	132	34
R22	Ridom SeqSphere+	50	463	19	132	34
R23	MLST2.0 (CGE tools)	50	463	19	132	34
R24	MLST (tsemann)	50	463	19	132	34
R25	MLST (tsemann)	50	463	19	132	34
R27	MLST2.0 (CGE tools)	50	463	19	132	34
R28	MLST (tsemann)	50	463	19	132	34
R30	MLST2.0 (CGE tools)	50	463	19	132	34
R31	MLST2.0 (CGE tools)	50	463	19	132	34
R32	Enterobase	50	463	19	132	34
R33	stringMLST, PubMLST	50	463	19	132	34
R34	Enterobase	50	463	19	132	34
R35	Enterobase	50	463	19	132	34
R38	MLST2.0 (CGE tools)	50	463	19	132	34
R39	Staramr tool using senterica_achtman_2 scheme on galaxy platform	50	463	19	132	34
R40	BioNumerics 8.1	50	463	19	132	34

Table S 7. Campylobacter species and methods used by the participants

Lab code	KmerFinder	Blast	Kraken	Other	TRING2C-1	TRING2C-4	TRING2C-7	TRING2C-9	TRING2C-10
R01				Ridom SeqSphere (Mash)	C. jejuni	C. jejuni	C. jejuni	C. jejuni	C. coli
R04			X		C. coli	C. jejuni	C. jejuni	C. jejuni	C. coli
R05	X				C. coli	C. jejuni	C. jejuni	C. jejuni	C. coli
R06	X		X		C. coli	C. jejuni	C. jejuni	C. jejuni	C. coli
R07	X				C. coli	C. jejuni	C. jejuni	C. jejuni	C. coli
R08				In-house campylobacter_Species ID (5 targets, Blast) run in SeqSphere+	C. coli	C. jejuni	C. jejuni	C. jejuni	C. coli
R09				PubMLST	C. coli	C. jejuni	C. jejuni	C. jejuni	C. coli
R12				SeqSphere	C. coli	C. jejuni	C. jejuni	C. jejuni	C. coli
R13				FastANI	C. coli	C. jejuni	C. jejuni	C. jejuni	C. coli
R14			X		C. coli	C. jejuni	C. jejuni	C. jejuni	C. coli
R15	X				C. coli	C. jejuni	C. jejuni	C. jejuni	C. coli
R17	X				C. coli	C. jejuni	C. jejuni	C. jejuni	C. coli
R19	X				C. jejuni	C. jejuni	C. jejuni	C. jejuni	C. jejuni
R20	X				C. coli	C. jejuni	C. jejuni	C. jejuni	C. coli
R21	X		X		C. coli	C. jejuni	C. jejuni	C. jejuni	C. coli
R22				rMLST, Ridom SeqSphere+ (Mash Distance)	C. coli	C. jejuni	C. jejuni	C. jejuni	C. coli
R23				Kraken2/bracken, incorporated in an in-house pipeline	C. coli	C. jejuni	C. jejuni	C. jejuni	C. coli
R24			X	rMLST	C. coli	C. jejuni	C. jejuni	C. jejuni	C. coli
R25	X				C. coli	C. jejuni	C. jejuni	C. jejuni	C. coli
R27	X			SpeciesFinder, ResFinder	C. coli	C. jejuni	C. jejuni	C. jejuni	C. coli
R28				rMLST	C. coli	C. jejuni	C. jejuni	C. jejuni	C. coli
R29	X				C. coli	C. jejuni	C. jejuni	C. jejuni	C. coli
R30				SpeciesFinder2.0 (CGE tools)	C. jejuni	C. jejuni	C. jejuni	C. jejuni	C. jejuni
R32				pubMLST	C. coli	C. jejuni	C. jejuni	C. jejuni	C. coli
R34	X				C. coli	C. jejuni	C. jejuni	C. jejuni	C. coli
R35		X			C. coli	C. jejuni	C. jejuni	C. jejuni	C. coli
R37				CLC Genomics Workbench Microbial genomic module Find Best matches using kmer spectra	C. coli	C. jejuni	C. jejuni	C. jejuni	C. coli
R38	X				C. coli	C. jejuni	C. jejuni	C. jejuni	C. coli
R39	X				C. coli	C. jejuni	C. jejuni	C. jejuni	C. coli

Table S 8. Campylobacter ST and methods used for identification by the participants.

Lab code	method	TRING2C-1	TRING2C-4	TRING2C-7	TRING2C-9	TRING2C-10
R01	Ridom SeqSphere	9263	257	7433	572	12073
R04	using pubmlst scheme as part of the in-house Bifrost setup	9263	257	7433	572	12073
R05	MLST2.0 (CGE tools)	9263	257	7433		
R06	MLST2.0 (CGE tools)	9263	257	7433	572	12073
R07	MLST2.0 (CGE tools)	9263	257	7433	572	12073
R08	PubMLST	9263	257	7433	572	12073
R09	Bionumerics 8.1	9263	257	7433	572	12073
R12	SeqSphere	9263	257	7433	572	12073
R13	PubMLST	9263	257	7433	572	12073
R14	MLST (tsemann)	9263	257	7433	572	12073
R15	MLST2.0 (CGE tools)	9263	257	7433	572	12073
R17	MLST2.0 (CGE tools)	9263	257	7433	572	12073
R19	MLST2.0 (CGE tools)					
R20	MLST2.0 (CGE tools)	9263	257	7433	572	12073
R21	MLST2.0 (CGE tools)	9263	257	7433	572	12073
R22	Ridom SeqSphere+	9263	257	7433	572	12073
R23	MLST2.0 (CGE tools)	9263	257	7433	572	830
R24	MLST (tsemann)	9263	257	7433	572	12073
R25	MLST (tsemann)	9263	257		572	12073
R27	MLST2.0 (CGE tools)	9263	257	7433	572	12073
R28	PubMLST	9263	257	7433	572	12073
R29	PubMLST	9263	257	7433	572	12073
R30	MLST2.0 (CGE tools)	9263	257	7433	572	12073
R32	MLST2.0 (CGE tools)	9263	257	7433	572	12073
R34	MLST2.0 (CGE tools)	9263	257	7433	572	12073
R35	PubMLST	9263	257	7433	572	12073
R37	CLC Genomics Workbench Microbial genomic module MLST typing	9263				12073
R38	MLST2.0 (CGE tools)	9263	257	7433	572	12073
R39	PubMLST	9263	257	7433	572	12073

8. Annex B

8.1. Supplementary gene tables for both organisms

This section contains tables with genes that were reported by some participants but not reported in any of the reference datasets. Gened involved in biocide resistance (such as *qacE*) are also in this section.

Table S 9. Additional genes reported in Salmonella strain TRING2S-1. Reference datasets, Res_Ref and AMR_Ref, are shaded grey. Participants are grouped based on database(s) used : Green – ResFinder, Blue – AMRFinderPlus with or without ResFinder, Yellow – CARD with or without any other database.

	ResFinder														AMRFinderPlus +/- ResFinder										CARD +/- others					
	Res_Ref	R15	R23	R25	R17	R30	R31	R27	R07	R33	R05	R18	R20	R38	AMR_Ref	R08	R35	R01	R10	R14	R04	R28	R21	R34	R39	R06	R02	R24	R32	R22	R40
ResFinder																														
AMRFinderPlus																					**									
CARD																														
Other database																													*	**
ant(3'')-Ia																												X		
blaCTX-M-1											X																			
cmlA1												X																		
fosA7																				X										
golS																										X				
mdsA*																			X											
mdsB																			X											
tet(M)						X																								

* GenBank
** BioNumerics 8.1

Table S 10. Additional genes reported in Salmonella strain TRING2S-2. Reference datasets, Res_Ref and AMR_Ref, are shaded grey. Participants are grouped based on database(s) used : Green – ResFinder, Blue – AMRFinderPlus with or without ResFinder, Yellow – CARD with or without any other database.

	ResFinder														AMRFinderPlus +/- ResFinder										CARD +/- others					
	Res_Ref	R15	R23	R25	R17	R30	R31	R27	R07	R33	R05	R18	R20	R38	AMR_Ref	R08	R35	R01	R10	R14	R04	R28	R21	R34	R39	R06	R02	R24	R32	R22	R40
ResFinder																														
AMRFinderPlus																					**									
CARD																														
Other database																													*	**
blaCARB-2																			X											
dfrA16																				X										
gacE								X																						
mdsA*																			X											
mdsB*																			X											
qacE		X	X										X							X										
qacEdelta1															X							X			X			X		X	
tet(D)																								X						
tet(G)								X																						
tet(M)								X																						

* GenBank
** BioNumerics 8.1

Table S 11. Additional genes reported in Salmonella strain TRING2S-4. Reference datasets, Res_Ref and AMR_Ref, are shaded grey. Participants are grouped based on database(s) used : Green – ResFinder, Blue – AMRFinderPlus with or without ResFinder, Yellow – CARD with or without any other database.

	ResFinder														AMRFinderPlus +/- ResFinder										CARD +/- others					
	Res_Ref	R15	R23	R25	R17	R30	R31	R27	R07	R33	R05	R18	R20	R38	AMR_Ref	R08	R35	R01	R10	R14	R04	R28	R21	R34	R39	R06	R02	R24	R32	R22	R40
ResFinder																														
AMRFinderPlus																					**									
CARD																														
Other database																													*	**
mdsB																			X							X				
mdsA																			X							X				
golS																										X				
qacE			X										X		X							X			X		X	X		
qacEdelta1																													X	

* GenBank
** BioNumerics 8.1

Table S 12. Additional genes reported in Salmonella strain TRING2S-7. Reference datasets, Res_Ref and AMR_Ref, are shaded grey. Participants are grouped based on database(s) used : Green – ResFinder, Blue – AMRFinderPlus with or without ResFinder, Yellow – CARD with or without any other database.

	ResFinder														AMRFinderPlus +/- ResFinder										CARD +/- others					
	Res_Ref	R15	R23	R25	R17	R30	R31	R27	R07	R33	R05	R18	R20	R38	AMR_Ref	R08	R35	R01	R10	R14	R04	R28	R21	R34	R39	R06	R02	R24	R32	R22	R40
ResFinder																														
AMRFinderPlus																					**									
CARD																														
Other database																													*	**
golS																										X				
mdsA																			X											
mdsB																			X											
qacE			X										X	X													X		X	
qacEdelta1															X				X		X	X			X	X	X	X		X	

* GenBank
** BioNumerics 8.1

Table S 13. Additional genes reported in Salmonella strain TRING2S-10. Reference datasets, Res_Ref and AMR_Ref, are shaded grey. Participants are grouped based on database(s) used : Green – ResFinder, Blue – AMRFinderPlus with or without ResFinder, Yellow – CARD with or without any other database.

	ResFinder														AMRFinderPlus +/- ResFinder										CARD +/- others					
	Res_Ref	R15	R23	R25	R17	R30	R31	R27	R07	R33	R05	R18	R20	R38	AMR_Ref	R08	R35	R01	R10	R14	R04	R28	R21	R34	R39	R06	R02	R24	R32	R22	R40
ResFinder																														
AMRFinderPlus																					**									
CARD																														
Other database																													*	**
aac(3)-II																									X					
aac(3)-IId																												X		
aac(6')-Ib (90,76 % identity)																							X							
aac(6')-Ib-cr5							X																							
ant(3'')-Ia																											X			
aph(3')-IIa																		X												
golS																										X				
mdsA																			X							X				
mdsB																			X							X				
qacE			X	X									X		X						X		X			X		X	X		
qacEdelta1																													X	

* GenBank
** BioNumerics 8.1

Table S 14. Additional genes reported in Campylobacter strain TRING2C-1. Reference datasets, Res_Ref and AMR_Ref, are shaded grey. Participants are grouped based on database(s) used : Green – ResFinder, Blue – AMRFinderPlus with or without ResFinder, Yellow – CARD with or without any other database.

	ResFinder														AMRFinderPlus +/- ResFinder										CARD +/- others						
	Res_Ref	R19	R15	R23	R25	R29	R17	R30	R27	R07	R05	R20	R01	R38	AMR_Ref	R14	R08	R35	R12	R04	R28	R21	R34	R39	R22	R13	R06	R24	R32	R09	R37
ResFinder																															
AMRFinderPlus																															
CARD																															
Other database																									[A]	[B]				[C]	[D]
ANT(6)-Ig																										X					
blaOXA-450						X	X					X																		X	
blaOXA-451						X	X					X																		X	
blaOXA-452						X	X					X																		X	
blaOXA-453						X	X					X																		X	
blaOXA-460																															X
blaOXA-489						X	X					X	X																	X	
blaOXA-61					X	X	X			X	X	X	X																	X	
cat-TC																															X

[A] GenBank
[B] NCBI + home-made database
[C] BioNumerics 8.1
[D] QMI-AR Peptide Marker Database (2021-08)

Table S 15. Additional genes reported in Campylobacter strain TRING2C-4. Reference datasets, Res_Ref and AMR_Ref, are shaded grey. Participants are grouped based on database(s) used : Green – ResFinder, Blue – AMRFinderPlus with or without ResFinder, Yellow – CARD with or without any other database.

	ResFinder														AMRFinderPlus +/- ResFinder										CARD +/- others						
	Res_Ref	R19	R15	R23	R25	R29	R17	R30	R27	R07	R05	R20	R01	R38	AMR_Ref	R14	R08	R35	R12	R04	R28	R21	R34	R39	R22	R13	R06	R24	R32	R09	R37
ResFinder																															
AMRFinderPlus																															
CARD																															
Other database																									[A]	[B]				[C]	[D]
blaOXA-460																															X
blaOXA-61						X					X																				
cmeR (?)																											X				

[A] GenBank
[B] NCBI + home-made database
[C] BioNumerics 8.1
[D] QMI-AR Peptide Marker Database (2021-08)

Table S 16. Additional genes reported in Campylobacter strain TRING2C-7. Reference datasets, Res_Ref and AMR_Ref, are shaded grey. Participants are grouped based on database(s) used : Green – ResFinder, Blue – AMRFinderPlus with or without ResFinder, Yellow – CARD with or without any other database.

	ResFinder														AMRFinderPlus +/- ResFinder										CARD +/- others						
	Res_Ref	R19	R15	R23	R25	R29	R17	R30	R27	R07	R05	R20	R01	R38	AMR_Ref	R14	R08	R35	R12	R04	R28	R21	R34	R39	R22	R13	R06	R24	R32	R09	R37
ResFinder																															
AMRFinderPlus																															
CARD																															
Other database																									[A]	[B]				[C]	[D]
aph(3'')-III				X																											
blaOXA-450						X	X					X																		X	
blaOXA-451						X	X					X																		X	
blaOXA-452						X	X					X																		X	
blaOXA-453						X	X			X		X																		X	
blaOXA-460																															X
blaOXA-489						X	X					X																		X	
blaOXA-595												X																			
blaOXA-61					X	X	X			X	X	X	X																	X	

[A] GenBank
[B] NCBI + home-made database
[C] BioNumerics 8.1
[D] QMI-AR Peptide Marker Database (2021-08)

Table S 17. Additional genes reported in Campylobacter strain TRING2C-9. Reference datasets, Res_Ref and AMR_Ref, are shaded grey. Participants are grouped based on database(s) used : Green – ResFinder, Blue – AMRFinderPlus with or without ResFinder, Yellow – CARD with or without any other database.

	ResFinder														AMRFinderPlus +/- ResFinder									CARD +/- others						
	Res_Ref	R19	R15	R23	R25	R29	R17	R30	R27	R07	R05	R20	R01	R38	AMR_Ref	R14	R08	R35	R12	R04	R28	R21	R34	R39	R22	R13	R06	R24	R32	R09	R37
ResFinder																															
AMRFinderPlus																															
CARD																															
Other database																									A	B				C	D
aph(3'')-III				X																											
blaOXA-450						X	X					X																		X	
blaOXA-451						X	X					X																		X	
blaOXA-452						X	X					X																		X	
blaOXA-453						X	X					X																		X	
blaOXA-460																															X
blaOXA-489						X	X			X		X																		X	
blaOXA-61					X	X	X			X	X	X	X																	X	
cmeR (?)																											X				

[A] GenBank
[B] NCBI + home-made database
[C] BioNumerics 8.1
[D] QMI-AR Peptide Marker Database (2021-08)

Table S 18. Additional genes reported in Campylobacter strain TRING2C-10. Reference datasets, Res_Ref and AMR_Ref, are shaded grey. Participants are grouped based on database(s) used : Green – ResFinder, Blue – AMRFinderPlus with or without ResFinder, Yellow – CARD with or without any other database.

	ResFinder														AMRFinderPlus +/- ResFinder									CARD +/- others						
	Res_Ref	R19	R15	R23	R25	R29	R17	R30	R27	R07	R05	R20	R01	R38	AMR_Ref	R14	R08	R35	R12	R04	R28	R21	R34	R39	R22	R13	R06	R24	R32	R09	R37
ResFinder																															
AMRFinderPlus																															
CARD																															
Other database																									A	B				C	D
aph(3")-III				X																											
blaOXA-450						X	X					X																		X	
blaOXA-451						X	X					X																		X	
blaOXA-452						X	X			X		X																		X	
blaOXA-453						X	X					X																		X	
blaOXA-460																															X
blaOXA-489						X	X					X																		X	
blaOXA-61					X	X	X			X	X	X	X																	X	

[A] GenBank
[B] NCBI + home-made database
[C] BioNumerics 8.1
[D] QMI-AR Peptide Marker Database (2021-08)

8.2. Supplementary point mutation tables for both organisms

This section contains tables with point mutations that were reported by some participants but not reported by the reference datasets.

Table S 19. Additional point mutations reported in Salmonella strain TRING2S-1. Reference datasets, Res_Ref and AMR_Ref, are shaded grey. Participants are grouped based on database(s) used : Green – PointFinder, Blue – AMRFinderPlus with or without PointFinder, Yellow – CARD with or without any other database.

	PointFinder														AMRFinderPlus +/- PointFinder														O
	Res_Ref	R15	R23	R25	R30	R31	R27	R07	R33	R05	R18	R20	R06	R38	AMR_Ref	R08	R04	R35	R01	R10	R14	R02	R17	R24	R32	R21	R22	R34	R39	R40
ResFinder																														
AMRFinderPlus																	*													
Other																														*
acrB F28L												X																		
gyrA								X						X																
parC						X																								
parC V261I												X																		
parC A620T												X																		
parC N395S												X																		
parC S469A												X																		

* BioNumerics 8.1

Table S 20. Additional point mutations reported in Salmonella strain TRING2S-2. Reference datasets, Res_Ref and AMR_Ref, are shaded grey. Participants are grouped based on database(s) used : Green – PointFinder, Blue – AMRFinderPlus with or without PointFinder, Yellow – CARD with or without any other database.

	PointFinder													AMRFinderPlus +/- PointFinder								
	Res_Ref	R15	R23	R25	R30	R31	R27	R33	R05	R18	R20	R06	R38	R04	R14	R02	R17	R24	R21	R34	R39
ResFinder																					
AMRFinderPlus														*							
acrB F28L											X										
acrB p.F28L					X																
acrB p.L40P					X																
pmrB H274Y											X										
acrB L40P											X										
pmrB p.H274Y					X																

* BioNumerics 8.1

Table S 21. Additional point mutations reported in Salmonella strain TRING2S-4. Reference datasets, Res_Ref and AMR_Ref, are shaded grey. Participants are grouped based on database(s) used : Green – PointFinder, Blue – AMRFinderPlus with or without PointFinder, Yellow – CARD with or without any other database.

	PointFinder									AMRFinderPlus +/- PointFinder				
	R23	R25	R30	R27	R33	R05	R18	R20	R06	R08	R14	R17	R24	R21
ResFinder														
AMRFinderPlus														
acrB F28L			X					X						
acrB L40P			X					X						
parC S469A			X					X						
parC A620T			X					X						
parC T255S			X					X						
parC N395S			X					X						

Table S 22. Additional point mutations reported in Salmonella strain TRING2S-7. Reference datasets, Res_Ref and AMR_Ref, are shaded grey. Participants are grouped based on database(s) used : Green – PointFinder, Blue – AMRFinderPlus with or without PointFinder, Yellow – CARD with or without any other database.

	PointFinder												AMRFinderPlus +/- PointFinder							
	Res_Ref	R15	R23	R25	R30	R27	R33	R05	R18	R20	R06	R38	R04	R14	R02	R17	R24	R21	R34	R39
ResFinder																				
AMRFinderPlus													*							
acrB F28L										X										
acrB L40P										X										
parC T255S										X										

Table S 23. Additional point mutations reported in Salmonella strain TRING2S-10. Reference datasets, Res_Ref and AMR_Ref, are shaded grey. Participants are grouped based on database(s) used : Green – PointFinder, Blue – AMRFinderPlus with or without PointFinder, Yellow – CARD with or without any other database.

	PointFinder									AMRFinderPlus +/- PointFinder				
	R23	R25	R30	R27	R33	R05	R18	R20	R06	R08	R14	R17	R24	R21
ResFinder														
AMRFinderPlus														
acrB F28L			X					X						
acrB L40P			X					X						
parC A620T			X					X						
parC N395S			X					X						
parC S469A			X					X						
parC T255S			X					X						

Table S 24. Additional point mutations reported in Campylobacter strain TRING2C-1. Reference datasets, Res_Ref and AMR_Ref, are shaded grey. Participants are grouped based on database(s) used: Green – PointFinder, Blue – AMRFinderPlus with or without PointFinder, Yellow – CARD with or without any other database.

	PointFinder															AMRFinderPlus +/- PointFinder												Other	
	Res_Ref	R37	R15	R19	R23	R25	R17	R30	R27	R07	R05	R20	R01	R06	R38	AMR_Ref	R08	R04	R35	R14	R12	R24	R32	R28	R21	R22	R34	R39	R13	R09
ResFinder																														
AMRFinderPlus																														
Other database																													*	**
cmeR S207G												X																		
rpsL A119T												X																		
cmeR A182T												X																		
cmeR V178I												X																		
cmeR T6I												X																		

* Card, Ncbi, home-made database
** BioNumerics 8.1

In sequence TRING2C-4, no point mutations were detected in the reference dataset. Participant R20, however, reported the following mutations: *gyrA* S22G, *gyrA* N203S, *gyrA* R285K, *gyrA* Q863 and *cmeR* P183R.

Table S 25. Additional point mutations reported in Campylobacter strain TRING2C-7. Reference datasets, Res_Ref and AMR_Ref, are shaded grey. Participants are grouped based on database(s) used : Green – PointFinder, Blue – AMRFinderPlus with or without PointFinder, Yellow – CARD with or without any other database.

	PointFinder																AMRFinderPlus +/- PointFinder												Other	
	Res_Ref	R37	R15	R19	R23	R25	R29	R17	R30	R27	R07	R05	R20	R01	R06	R38	AMR_Ref	R08	R04	R35	R14	R12	R24	R32	R28	R21	R22	R34	R39	R13	R09
PointFinder																															
AMRFinderPlus																															
Other database																													*	**
gyrA S22G													X																		
cmeR P183R													X																		
cmeR S207G													X																		
gyrA N203S													X																		
cmeR T6I													X																		

* Card, Ncbi, home-made database
** BioNumerics 8.1

Table S 26. Additional point mutations reported in Campylobacter strain TRING2C-9. Reference datasets, Res_Ref and AMR_Ref, are shaded grey. Participants are grouped based on database(s) used : Green – PointFinder, Blue – AMRFinderPlus with or without PointFinder, Yellow – CARD with or without any other database.

	PointFinder																AMRFinderPlus +/- PointFinder												Other	
	Res_Ref	R37	R15	R19	R23	R25	R29	R17	R30	R27	R07	R05	R20	R01	R06	R38	AMR_Ref	R08	R04	R35	R14	R12	R24	R32	R28	R21	R22	R34	R39	R13	R09
PointFinder																															
AMRFinderPlus																															
Other database																														*	**
G57T mutation in blaOXA193 promoting region																														X	

* Card, Ncbi, home-made database
** BioNumerics 8.1

No additional mutations were reported for sequence TRING2C-10.

[Catalogue number]

Publications Office
of the European Union

ISBN [number]