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

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

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1. Background and aim

This report presents the organisation, execution and results of the third *in silico* inter-laboratory ring trial of bioinformatics pipelines for prediction of AMR genes in antimicrobial resistant *Salmonella* and *Campylobacter* (RingTrial3-WGS-AMR, hereafter referred to as RingTrial3, or RT3), the third out of three planned ring trials, organized by Statens Serum Institut (SSI) in the FWD AMR-RefLabCap project in years 2022-24.

The third ring trial was organised according to the work plan (Deliverable T1.7), as well as using information from the first two ring trials. 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 detect antimicrobial resistance genes and point mutations (PMs) 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) and follow the recommendations for prediction of resistance traits. Participation in the RingTrial3 enabled the laboratories to identify strengths and weaknesses in their analytical setup and implement improvements, if needed.

DNA sequences (paired end Illumina reads and SPAdes assemblies) from four *Salmonella* and four *Campylobacter* isolates were included in this RingTrial3. Forty-one participants were invited, 36 accepted the invitation and 32 submitted results. The participants represented a total of 27 countries, including 9 priority countries.

2. Materials and methods

2.1. Phenotypic testing

The isolates 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 cut-off values (ECOFF), when available (1). MIC determination was performed following the harmonised EU AST protocol using microbroth dilution method with EUVSEC3 TREK panels from Thermo Scientific, Denmark for *Salmonella* 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, Ampicillin, Azithromycin, Cefotaxime, Ceftazidime, Chloramphenicol, Ciprofloxacin, Colistin, Gentamicin, Meropenem, Nalidixic acid, Sulfamethoxazole, Tetracycline, Tigecycline, and Trimethoprim. For *Campylobacter*, the panels included Chloramphenicol, Ciprofloxacin, Ertapenem, Erythromycin, Gentamicin and Tetracycline. The results of phenotypic testing are shown in Table 1 and Table 2. The phenotypic interpretation to WT/NWT was done if an ECOFF was available. The selection of antimicrobials tested was based on the list of antimicrobials set in the harmonised EU AST protocol (1), recommended by the ECDC.

2.2. Sequence characterization

The sequences used in this RingTrial3 represent isolates with a wide variety of antimicrobial resistance markers. The genotypic and phenotypic antimicrobial resistance features of each isolate/sequence are shown in Table 1 and Table 2.

It is only possible to derive a phenotype from WGS-based data if the applied database offers the opportunity to predict a phenotype based on the derived sequences (genes and

or PM). Additionally, the ECOFFs, set by the European Committee on Antimicrobial Susceptibility Testing (EUCAST), have to be available for the antimicrobial in question in order to determine the laboratory-based susceptibility of an isolate.

In most cases in this EQA, it was possible to compare the phenotypic predictions for the tested antimicrobials with the laboratory-established phenotypes for the test isolates. However, some isolates included in this EQA harbour genes or PMs that confer resistance towards antimicrobials that were not tested phenotypically in the laboratory. Additionally, there are phenotypes for which the genetic determinants have not been elucidated.

For these reasons it was not possible to determine the correlation between phenotype and genotype for several antimicrobials, for example Rifampicin and Erythromycin genotype in *Salmonella* or Streptothricin genotype in *Campylobacter*.

The known phenotype-genotype correlations are described below in each Table.

Table 1. Genotypic and phenotypic characteristics of Salmonella isolates selected for the RingTrial3

Isolate	RING3S-1	RING3S-2	RING3S-3	RING3S-4
Serotype	Corvallis	Typhimurium	Kentucky	4,5,12:i:-
ST	1541	19	198	34
Genes[A]	*aph(3'')-Ib, aph(6)-Id, qnrS1, sul2, tet(A)*	*aadA2, ant(2'')-Ia, blaCTX-M-9, catA1, dfrA16, qnrA1, sul1, tet(A)*	*aadA1, aadA2, aph(3')-Ia, blaTEM-57, cmlA1, dfrA12, dfrA5, floR, fosA4, mph(A), sul1, sul3, tet(A)*	*aac(3)-IIg, aac(6')-IIc, aac(6')-Ib3, aadA2, aph(3'')-Ib, aph(3')-Ia, aph(6)-Id, arr, blaSHV-12, blaTEM-1, dfrA19, ere(A), qnrB2, sul1, sul2, tet(B), tet(D)*
PMs[A]			*gyrA_D87G, gyrA_S83F, parC_S80I*	
NWT Phenotypes[B]	CIP, TCY	AMP, CTX, CHL, CIP, GEN, NAL, TCY, TMP	AMP, AZM, CHL, CIP, NAL, TCY, TMP	AMI, AMP, CTX, CAZ, CIP, GEN, NAL, TCY, TMP

A According to AMRFinderPlus version 3.11.26 (database version 2023-11-15.1)
B Abbreviations of antimicrobials: AMI (Amikacin), AMP (Ampicillin), AZM (Azithromycin), Cefotaxime (CTX), Ceftazidime (CAZ), CHL (Chloramphenicol), CIP (Ciprofloxacin), GEN (Gentamicin), NAL (Nalidixic acid), TCY (Tetracycline), TMP (Trimethoprim)

Sequences RING3S-2, RING3S-3 and RING3S-4 contain beta lactam genes such as *blaCTX-M-9*, *blaTEM-57*, *blaSHV-12* and *blaTEM-1*, which confer resistance to cephalosporins such as Cefepime, Cefotaxime, Ceftazidime and penicillins such as Ampicillin (2).

The presence of genes *sul1*, *sul2* and *sul3* in all four sequences indicates Sulfamethoxazole resistance (3), and this was confirmed by high MIC values obtained in the laboratory tests for this antimicrobial. However, due to the lack of an established ECOFF for Sulfamethoxazole, it would be incorrect to state that the isolate behind the sequence is phenotypically resistant. Genes *tet(A)*, *tet(B)* and *tet(D)*, present in all sequences, are responsible for resistance to Tetracycline (3).

Chloramphenicol resistance in sequences RING3S-2 and RING3S-3 is mediated by genes *catA1*, coding for chloramphenicol acetyl transferase, and efflux pumps encoded by *floR* and *cmlA1* (3)(4)(5). Gene variants *dfrA5*, *dfrA12*, *dfrA16* and *dfrA19* in sequences RING3S-2 to RING3S-4 are responsible for Trimethoprim resistance in these samples and are associated with Class I or Class II integrons, plasmids or Salmonella Genomic Island 1 or 2 (6)(7).

Resistance to fluoroquinolones, such as Ciprofloxacin, can be linked to genes *qnrS1*, *qnrA1* and *qnrB2* in sequences RING3S-1, RING3S-2 and RING3S-4, respectively, whereas in sequence RING3S-3, all three PMs, in *gyrA* and *parC*, likely contribute to Ciprofloxacin (8) and Nalidixic acid resistance. However, laboratory-based resistant phenotype to Nalidixic acid in isolates RING3S-2 and RING3S-4 is unclear, as they do not harbour the corresponding PMs. It has been shown that the presence of the *qnr* gene alone does not mediate resistance to Nalidixic acid, as opposed to the presence of one or more PMs (9). Additionally, it is unclear whether the *parC* T57S substitution contributes to

ciprofloxacin resistance, as the presence of this mutation was previously observed mostly in *Salmonella* strains with ciprofloxacin MIC values ≤ 0.06 mg/L (10).

All three test isolates have a number of aminoglycoside genes, including phosphotransferases (*aph*), acetyltransferases (*aac*) and nucleotidyl transferases (*aad* and *ant*). Of these, the genes belonging to the two latter types can be responsible for Gentamicin resistance (3), which was observed in sequences RING3S-2 and RING3S-4. Phenotypic resistance to aminoglycoside Amikacin is observed in sequence RING3S-4, which is likely mediated by gene *aac(6')-Ib3* (11).

Gene mph(A), present in sequence RING3S-3, leads to Azithromycin resistant phenotype (2).

Sequence RING3S-4 harbours genes *arr*, associated with Rifamycin resistance (12); and *ere(A)*, that are associated with Erythromycin resistance (2). These two antimicrobials, however, were not present among the ones tested in the laboratory, therefore, it was not possible to confirm the predicted phenotype.

Table 2. Genotypic and phenotypic characteristics of Campylobacter isolates selected for the RingTrial3

Isolate	**RING3C-1**	**RING3C-2**	**RING3C-3**
Species	*C. jejuni*	*C. coli*	*C. coli*
ST	464	12073	828
Genes[A]	*blaOXA, tet(O)*	*aad9, aadE, aph(3')-IIIa, blaOXA-193, catA, erm(B), sat4, tet(O)*	*aac(6')-Im, aph(2'')-IIa, aph(3')-IIIa, blaOXA-193, sat4, tet(O)*
PMs[A]	*50S_L22_A103V, gyrA_T86I*	*gyrA_T86I, rpsL_K43R*	*23S_A2075G, gyrA_T86I*
NWT Phenotypes[B]	CIP, TCY	CHL, CIP, ERY, TCY	CIP, ERY, GEN, TCY

A According to AMRFinderPlus
B Abbreviations of antimicrobials: CHL (Chloramphenicol), CIP (Ciprofloxacin), ETP (Ertapenem), ERY (Erythromycin), GEN (Gentamicin), NAL (Nalidixic acid), TCY (Tetracycline)

Initially, four *Campylobacter* sequences were shared with the participants, however, sequence RING3C-4 was found to give ambiguous results (indicating either contamination or a hybrid sequence) and therefore, was removed from this analysis.

In the three *Campylobacter* sequences, Ciprofloxacin (fluoroquinolone) resistance is mediated through the *gyrA* T86I PM (13), present in each isolate. The *23S* A2075G substitution in sample RING3C-3 is responsible for Erythromycin (macrolide) resistance (13). Chloramphenicol (phenicol) resistance in sequence RING3C-2 is due to the presence of the *catA* gene (14). All samples harbour the *tet(O)* gene, which mediates Tetracycline resistance. Gentamicin (aminoglycoside) NWT phenotype identified in sample RING3C-3 is likely due to the presence of the *aph(2'')-IIa* gene.

Point mutations in the L22 subunit of the 50S ribosomal protein are known to confer high level resistance to Erythromycin in other species (15). However, in *Campylobacter*, mutations such as *50S* L22 A103V, present in sequence RING3C-1, are seen both in Erythromycin sensitive and resistant isolates and have been shown not to contribute to resistance to this antimicrobial (16).

The genes *aad9* and *aadE* in sample RING3C-2 likely results in resistance to Streptomycin (aminoglycoside) (17)(18), together with the *rpsL* K43R substitution in the same sample (16). Resistance to this antimicrobial could not be confirmed phenotypically in this RingTrial (see paragraph 2.1 for details). Similarly, the *gyrA* T86I substitution would likely result in resistance to Nalidixic acid (quinolone)(13) in all sequences, the presence of gene *aph(3')-IIIa* in sequences RING3C-2 and RING3C-3 in amikacin or kanamycin resistance and the presence of gene *sat4* in RING3C-3 in streptothricin resistance (18). These, however, could not be confirmed phenotypically in the present study.

Two different variants of the *blaOXA* genes are present in all samples, without a phenotypic confirmation. The expected phenotype would be resistance to beta-lactam antibiotics. The relation between the presence of *blaOXA* genes in *Campylobacter* spp. to a phenotype is complex. It is not the presence of the gene itself, but the presence of a G to T mutation in the promoter region of the *blaOXA-61* gene that is responsible for resistance to Ampicillin (17)(19)(20).

2.3. WGS analysis by the Ring Trial provider

DNA from *Salmonella* and *Campylobacter* isolates was sequenced using paired-end Illumina sequencing. The quality of these 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 January 2024 for the presence of antimicrobial resistance genes and PMs by querying two different databases: ResFinder and AMRFinderPlus. The versions used and the results obtained with the two approaches, shown in Table 3, will be referred to as “reference datasets” in the report.

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

	AMR gene detection			**Point mutation identification**		
Dataset	**Database**	**Tool**	**Input**	**Database**	**Tool**	**Input**
Res_Ref	ResFinder (21) (2023-04-12)	ResFinder (v. 4.4.2)	Raw reads (fastq)	PointFinder (22) (2023-05-03)	ResFinder (v. 4.4.2)	Raw reads (fastq)
AMR_Ref	AMRFinderPlus (23) (2023-11-15.1)	AMRFinderPlus (v. 3.11.26)	SPAdes assembly (fasta protein)	AMRFinderPlus (2023-11-15.1)	AMRFinderPlus (v. 3.11.26)	SPAdes assembly (fasta nucleotide)

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

Sequences from all isolates, 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://rambollxact.com).

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, PMs, as well as

the serotype and species for *Salmonella* and *Campylobacter*, respectively. Questions in this part also included identity and coverage cut-offs used for identifying genes and PMs, as well as questions about the approach used for reporting. The second part was for reporting AMR genes and PMs. It was possible to select multiple genes from a list of genes in alphabetical order and to report a gene in a free text field, in case it was not present on the default list. For reporting of PMs, 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.

Twenty-seven participants reported results for *Salmonella* and *Campylobacter*, respectively (22 reporting for both *Salmonella* and *Campylobacter* and five laboratories reporting for *Salmonella* only and *Campylobacter* only). The participating countries were Austria, Belgium, Croatia, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Moldova, the Netherlands, Norway, Portugal, Serbia, Slovakia, Slovenia and Sweden. The participating laboratories were randomly assigned anonymised laboratory codes and these codes were used for identification of laboratories in this RingTrial report.

3. Salmonella results

3.1. AMR gene and PMs detection methods used

3.1.1. Tools and databases used for AMR gene detection

All twenty-seven 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 and what versions of tools and databases were used.

Fourteen participants applied one tool and one database, eight participants a combination of two tools and two databases and three participants a combination of three tools and three databases. Of the remaining two participants, one used a combination of two tools and one database and one a combination of four tools and three databases (Table S1). Taken all variables (tools/databases/versions/inputs/thresholds/gene reporting strategies) into account, overall, 26 unique combinations were used by 27 participants. It was observed that participants often were reporting the same version for both tool and the database, and there were often inconsistencies in the reporting on the inputs used (Table S1).

The most commonly used tool was ResFinder (20 participants), followed by AMRFinderPlus (15 participants). An overview of all tools used is available in Figure 1.

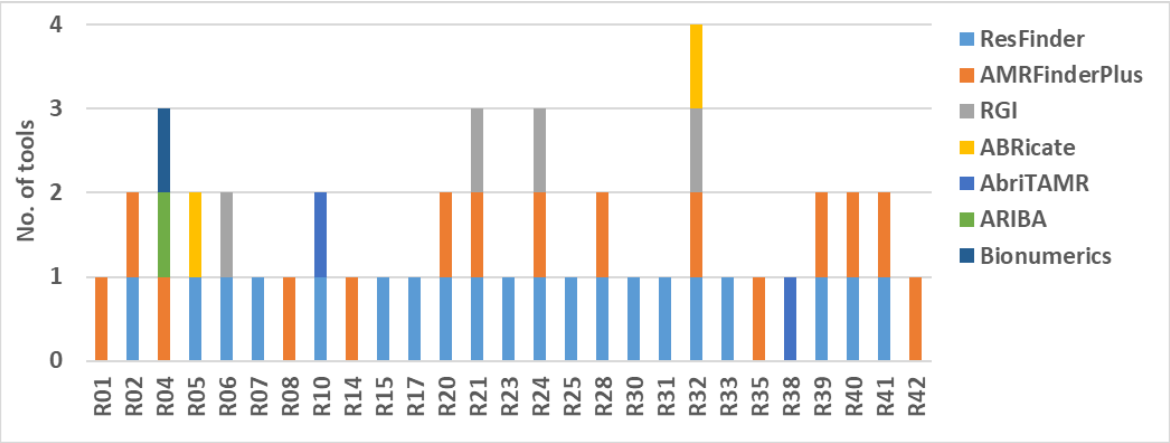

Figure 1. An overview of tools used by 27 participants for AMR gene detection in Salmonella

The ResFinder database was used by 21 participants and the AMRFinderPlus database by 17 participants. The CARD database was used by four participants and BioNumerics by one participant. The participants also indicated how they reported AMR genes. Fourteen participants reported all genes detected by the method in use, eleven reported a subset of genes based on experience/knowledge/literature and two participants reported a consensus list of genes (common genes present in all databases used) (Figure 2). Additionally, participant R40 indicated that they reported all genes found with AMRFinderPlus, supplemented with CGE where identity was lower than 99%. Participant R32 stated that reporting was based on five tools (Abricate, two versions of ResFinder, AMRFinder and CARD), where they reported a gene, if it was present in three tools out of five with at least 95% identity (Table S1).

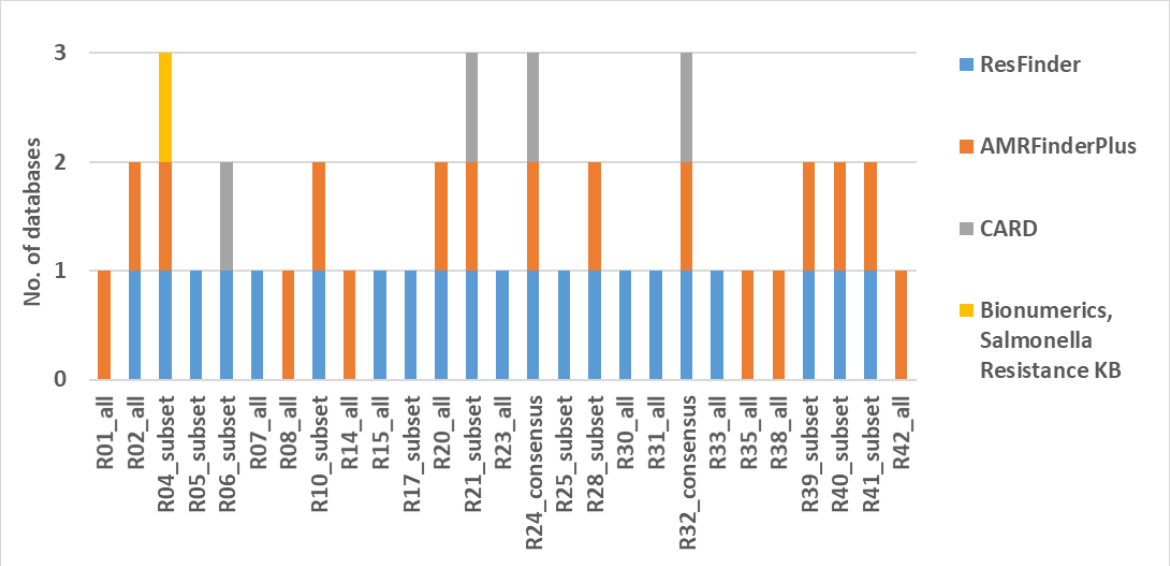

Figure 2. An overview of databases used by 27 participants for AMR gene detection in Salmonella. The horizontal labels indicate the participant ID and how they reported AMR genes: all – all genes detected by the method in use/from all databases, subset – a subset of genes based on experience/knowledge/literature, consensus – a consensus list of genes (common genes present in all databases used).

3.1.2. Tools and databases used for point mutation detection

All 27 participants reported the tools, the databases, and the inputs that they used for detection of point mutations and also how the point mutations were reported. In addition, they reported the versions of tools and databases used.

Eighteen participants used one tool and one database, seven participants - a combination of two tools and two databases, one - a combination of three tools and two databases, and one a combination of three tools and three databases (Figure 3). Taken all variables (tools/databases/versions/inputs/thresholds/reporting strategies) into account,

overall, 26 unique combinations were used by 27 participants. It was observed that participants often were reporting the same version for both tool and the database and there were often inconsistencies on the reporting of used inputs (Table S2).

PointFinder was the preferred tool, being used by 19 participants, either alone or in combination with another tool. AMRFinderPlus was the second most common tool and was used by 14 participants (Figure 3).

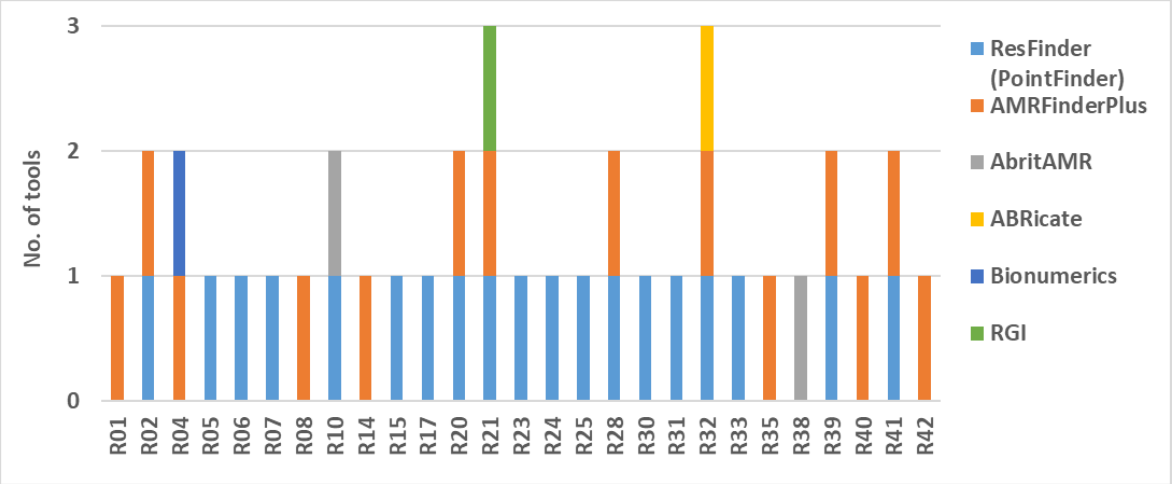

Figure 3. An overview of tools used by 27 participants for point mutations detection in Salmonella

The ResFinder database was used by 19 participants and the AMRFinderPlus database was used by 16 participants. The CARD database and BioNumerics *Salmonella* Resistance KB were used by one participant each (Figure 4).

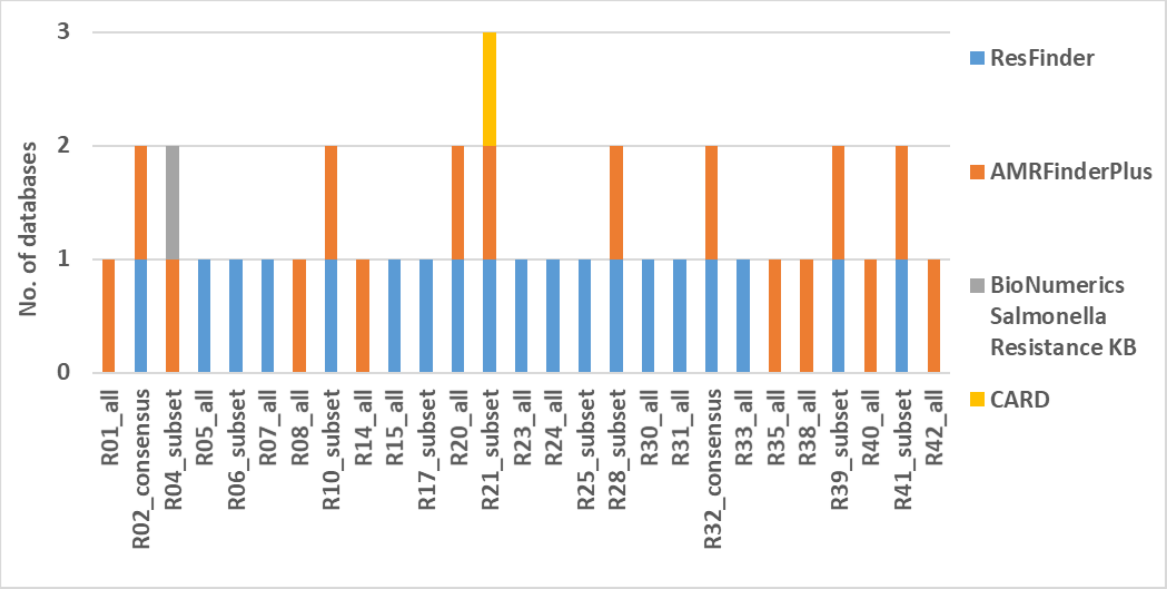

Figure 4. An overview of databases used by 27 participants for point mutation detection in Salmonella. The horizontal labels indicate the participant ID and how they reported point mutations: all – all point mutations detected by the method in use/from all databases, subset – a subset of point mutations based on experience/knowledge/literature, consensus – a consensus list of point mutations (common point mutations present in all databases used).

3.2. Serotypes and STs reported

3.2.1. Serotyping methods and serotypes

Twenty-five out of 27 participants used SeqSero for serotype identification, either alone or in combination with other tools. The other tools are visible in Figure 5 below.

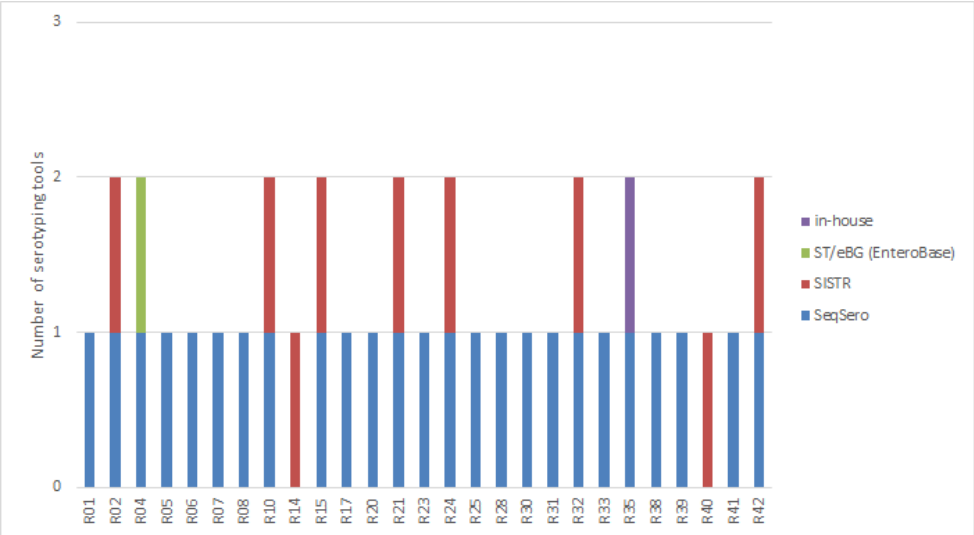

Figure 5. An overview of tools used by 27 participants for Salmonella serotyping

All 27 participants reported the same serotypes for all four *Salmonella* sequences: RING3S-1 (Corvallis), RING3S-2 (Typhimurium), RING3S-3 (Kentucky) and RING3S-4 (monophasic Typhimurium), in concordance with the reference dataset (Table S5). Two participants, R01 and R17, reported RING3S-1 serotype as “Corvallis or Chailey”.

3.2.2. MLST methods and STs

The most common method used for MLST reporting was MLST2.0 from CGE tools, used by 9 participants. Seven participants used MLST (tsemann), six participants used SeqSphere, two Enterobase, two used an in-house pipeline and 1 used SRST2 (Table S6).

All twenty-seven participants reported the correct STs for all sequences: RING3S-1 (ST1541), RING3S-2 (ST19), RING3S-3 (ST198) and RING3S-4 (34).

3.3. AMR genes and PMs reported for Salmonella sequences

The genes identified by the Ring Trial provider using two different tools and databases, Res_Ref and AMR_Ref, and the genes identified by the participants are presented for each sequence in the following paragraphs.

For each gene and PM table, the concordance of the reported results among the participants was calculated as the percentage of the total number of participants that reported the same genes or PMs for a given DNA sequence. If a participant deemed the quality of the sequence to be insufficient for reporting genes or PMs for a given sequence, the participant was not included in the calculation for that sequence.

An overview of the observed discrepancies between the two reference datasets in all *Salmonella* sequences is presented in Table 4 below.

Table 4. Suggested explanation of differences between reference datasets Res_Ref and AMR_Ref, observed in gene and PM reporting for all Salmonella test sequences. "X" indicates in which database the gene is present.

Gene / PM	Res_Ref	AMR_Ref	Possible explanation	Reference / comment
aac(3)-IIg		X	Variant absent from ResFinder database	RING3S-4
aac(6')-Iaa	X		Gene absent from the AMRFinderPlus database. Does not contribute to aminoglycoside resistance in Salmonella.	(24) RING3S-1-4
ant(3'')-Ia	X		Gene present in both AMRFinderPlus and ResFinder. In sequence RING3S-3 in the AMR_Ref dataset it was reported under the alternative name, *aadA1*.	RING3S-3
arr		X	ResFinder database contains 10 variants of the arr gene but the gene from sequence RING3S-4 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.	RING3S-4
blaTEM-1		X	Gene *blaTEM-1* present in AMRFinderPlus database	RING3S-3, RING3S-4
blaTEM-1B	X		Variant *blaTEM-1B* present in ResFinder database	RING3S-3, RING3S-4
parC T57S	X		Mutation present only in ResFinder database	RING3S-1, RING3S-3

3.3.1. Sequence RING3S-1

For RING3S-1, a high concordance (above 90%) was observed for all genes present in both databases (Table 5). Participant R06 did not report genes *aph(3'')-Ib* and *aph(6)-Id*, perhaps as a consequence of their reporting strategy – reporting a subset of genes. The strategy of participant R01, on the other hand, was to report all genes detected by the method used and yet *aph(3'')-Ib* was not reported by this participant.

Table 5. AMR genes reported in Salmonella sequence RING3S-1. Reference datasets, Res_Ref and AMR_Ref, are shaded grey. The letter "X" indicates the detection of a specific gene. 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 the 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	R05	R07	R15	R17	R23	R25	R30	R31	R33	AMR_Ref	R01	R08	R14	R35	R38	R42	R02	R10	R20	R28	R39	R40	R41	R04	R06	R21	R24	R32	% concordance
ResFinder																														
AMRFinderPlus																														
CARD																														
Other																									*					
aac(6')-Iaa	X	X	X	X		X	X	X		X											X					X	X	X		41
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	93
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	96
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	100
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	100
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	X	100

* Bionumerics, Salmonella Resistance KB, version 2021.04.12

As expected, the *parC* T57S mutation was reported only by participants using the PointFinder database (alone or with other databases), with exception of three participants, R07, R10 and R17 (Table 6). The two latter participants reported a subset of point mutations, based on literature or knowledge, but the approach of participant R07 was to report all point mutations. It is therefore unclear why this mutation was not reported.

Table 6. Point mutations (PMs) reported in Salmonella sequence RING3S-1. Reference datasets, Res_Ref and AMR_Ref, are shaded grey. The letter "X" indicates the detection of a specific PM. Participants are grouped based on database(s) used: Green – PointFinder, Blue – AMRFinderPlus with or without PointFinder, Yellow – Other (other database(s), specified below the table). Percentage concordance is based on the 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		% concordance
	Res_Ref	R05	R06	R07	R15	R17	R23	R24	R25	R30	R31	R33	AMR_Ref	R01	R08	R14	R35	R38	R40	R42	R02	R10	R20	R28	R32	R39	R41	R04	R21	
PointFinder																													
AMRFinderPlus																													
Other																											*	**	
parC T57S	X	X	X		X		X	X	X	X	X	X									X		X	X	X	X	X	X	63

* Bionumerics, Salmonella Resistance KB version 2021.04.12
** RGI- 6.0.3

3.3.2. Sequence RING3S-2

In sequence RING3S-2, seven out of nine genes were reported by more than 90% of participants. Gene *aadA2* was not reported by 4 participants. All these participants, R05, R06, R10 and R40 used a subset of genes in their reporting.

Table 7. AMR genes reported in Salmonella sequence RING3S-2. Reference datasets, Res_Ref and AMR_Ref, are shaded grey. The letter "X" indicates the detection of a specific gene. 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 the 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	R05	R07	R15	R17	R23	R25	R30	R31	R33	AMR_Ref	R01	R08	R14	R35	R38	R42	R02	R10	R20	R28	R39	R40	R41	R04	R06	R21	R24	R32	
ResFinder																															
AMRFinderPlus																															
CARD																															
Other																									*						
aac(6')-Iaa	X	X	X	X		X	X	X		X											X					X	X	X		41
aadA2	X		X	X	X	X	X[A]	X	X	X	X	X	X	X	X	X	X	X		X	X	X		X	X		X	X	X	81
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	100
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	100
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	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	96
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	96
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	100
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	93

* Bionumerics, Salmonella Resistance KB, version 2021.04.12
[A] reported as *aadA2b*

No point mutations were present or reported in sequence RING3S-2.

3.3.3. Sequence RING3S-3

In sequence RING3S-3, 11 out of 17 genes were reported by more than 90% of the participants. Gene *blaTEM-1B* was reported only by one participant and by 4 participants as *blaTEM-1* (Table 8). In the Res_Ref dataset, the gene *blaTEM-1B* was detected with 90.24% identity and coverage when using reads. Since most participants in the green category (ResFinder only) applied the identity cut-off of 90% (1 participant used 80%), the gene was expected to be reported by the participants that used ResFinder. The versions of the database used varied among participants in the green category, from 2.2.1 to 4.5.0. It could be speculated that certain local settings could have affected the result for these participants.

Three participants from the blue group reported the gene *blaTEM-1*. Participant R14 used the same assembly file as was used in the AMR_Ref dataset and the exact same version of the tool and database, but reported the gene, whereas it was not detected in AMR_Ref dataset. The coverage cut-off applied in AMR_Ref was 50% compared to 90% used by R14.

Eight out of 27 participants did not report the *aadA2* gene. In the AMR_Ref dataset, this gene was detected with 73.8% coverage (and 100% identity). Out of the six participants from the blue category, that used AMRFinderPlus only, participants R01 and R38 used a coverage cut-off of 100%, which can explain why they did not report the gene. Of the four participants who did report the gene, three (R08, R35 and R42) used a coverage cut-off of 50% and one (R14) of 90%. It is unclear how R14 could have reported the gene with 90% coverage cut-off when the same assembly as in AMR_Ref was used. In the Res_Ref dataset, the coverage for *aadA2* was correspondingly low (73%). Out of nine participants in the green group (ResFinder only), two that did not report the gene (R05 and R25) used a coverage cut-off of 60%, but performed their own assemblies, which potentially could have affected the result.

The *mph(A)* gene was also detected in AMR_Ref and Res_Ref datasets with lower coverage (64%). Correspondingly, two participants in the blue group that only used AMRFinderPlus and applied coverage cut-off of 100%, did not report this gene. In the green category, only one participant did not report this gene.

Table 8. AMR genes reported in Salmonella sequence RING3S-3. Reference datasets, Res_Ref and AMR_Ref, are shaded grey. The letter "X" indicates the detection of a specific gene. 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 the 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	R05	R07	R15	R17	R23	R25	R30	R31	R33	AMR_Ref	R01	R08	R14	R35	R38	R42	R02	R10	R20	R28	R39	R40	R41	R04	R06	R21	R24	R32	% concordance
ResFinder																														
AMRFinderPlus																														
CARD																														
Other																									*					
aac(6')-Iaa	X	X	X	X		X	X	X		X											X					X	X	X		41
aadA1	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
ant(3'')-Ia	X			X	X	X				X																	X			100
aadA2	X		X	X	X	X		X	X	X	X		X	X	X		X		X	X	X	X		X	X		X	X		70
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	100
blaTEM-1									X					X						X					X					
blaTEM-1B	X			X																										19
blaTEM-57	X	X	X	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
cmlA1	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	100
dfrA12	X	X	X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X	93
dfrA5	X	X	X	X	X	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	96
fosA4	X	X	X	X	X	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
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	78
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	96
sul3	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	100
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	96

* Bionumerics, Salmonella Resistance KB, version 2021.04.12

Four PMs in two genes were reported in the Res_Ref dataset and three PMs in the AMR_Ref dataset, consistent with what is available in the corresponding databases (see Table 4). Participant R01 reported not finding any mutations in RING3S-3, despite analysing the same assembly as used in AMR_Ref (Table 9).

Table 9. Point mutations (PMs) reported in Salmonella sequence RING3S-3. Reference datasets, Res_Ref and AMR_Ref, are shaded grey. The letter "X" indicates the detection of a specific PM. Participants are grouped based on database(s) used: Green – PointFinder, Blue – AMRFinderPlus with or without PointFinder, Yellow – Other (other database(s), specified below the table). Percentage concordance is based on the 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	R05	R06	R07	R15	R17	R23	R24	R25	R30	R31[A]	R33	AMR_Ref	R01	R08	R14	R35	R38	R40	R42	R02	R10	R20	R28	R32	R39	R41	R04	R21	% concordance
PointFinder																													
AMRFinderPlus																													
Other																											*	**	
gyrA D87G	X	X	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
gyrA S83F	X	X	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
parC T57S	X	X	X	X	X	X	X	X	X	X		X									X		X	X	X	X	X	X	67
parC S80I	X	X	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

* Bionumerics, Salmonella Resistance KB version 2021.04.12
** RGI- 6.0.3
[A] Participant R31 reported only genes (gyrA, parC), without specifying mutations (see Table S10)

3.3.4. Sequence RING3S-4

In sequence RING3S-4, 10 out of 18 genes were reported by more than 90% of the participants. Most of the differences in reporting among participants could be attributed to the presence or absence of certain genes in the corresponding databases (see Table 4 for details). Several genes in this sequence had lower coverage in AMRFinderPlus, for example, *aph(3'')-Ib* (74.5%), *ere(A)* (84.8%) and *sul2* (72.7%). In ResFinder, when assemblies were used, the same three genes had similarly lower coverage. Participants that used high coverage cut-offs, for example R01 and R38 for AMRFinderPlus (100% coverage cut-off), did not report these genes. However, participant R05 that used ResFinder, also did not report those genes, in spite of reporting a coverage cut-off of 60%.

Table 10. AMR genes reported in Salmonella sequence RING3S-4. Reference datasets, Res_Ref and AMR_Ref, are shaded grey. The letter "X" indicates the detection of a specific gene. 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 the 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	R05	R07	R15	R17	R23	R25	R30	R31	R33	AMR_Ref	R01	R08	R14	R35	R38	R42	R02	R10	R20	R28	R39	R40	R41	R04	R06	R21	R24	R32	% concordance
ResFinder																													
AMRFinderPlus																													
CARD																													
Other																									*				
aac(3)-IIg											X	X	X	X	X	X	X		X	X	X	X	X	X	X	X			X	56
aac(6')-Iaa	X	X	X	X		X	X	X		X											X					X	X	X		41
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	96
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	100
aadA2	X		X	X	X	X	X[A]	X	X	X	X	X	X	X	X	X	X	X		X	X	X	X	X	X		X	X	X	89
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	96
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	78
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	85
arr											X	X	X	X	X	X	X		X	X	X	X	X	X	X	X			X	X	56
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	100
blaTEM-1									X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
blaTEM-1B	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	100
ere(A)	X		X	X	X	X	X	X	X	X	X		X	X	X		X			X	X	X		X			X	X	X	70
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	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	100
sul2	X		X	X	X	X	X	X	X	X	X		X	X	X		X		X	X	X	X		X	X		X	X	X	78
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	96
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	100

* Bionumerics, Salmonella Resistance KB, version 2021.04.12
[A] reported as aadA2b

No point mutations were present or reported in sequence RING3S-4.

4. Campylobacter results

4.1. AMR gene and PMs detection methods used

4.1.1. Tools and databases used for gene detection

All 27 participants were asked to report 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. In addition, they were asked to report the versions of tools and databases used.

Sixteen participants applied one tool and one database, six participants applied a combination of two tools and two databases, two participants a combination of three tools and three databases. Of the remaining three participants, one used a combination of one tool and three databases, one a combination of two tools and one database, and one participant – a combination of four tools and three databases.

Taken all variables (tools/databases/versions/inputs/thresholds/gene reporting strategies) into account, overall, 26 unique combinations were used by 27 participants. It was observed that participants often were reporting the same version for both tool and the database, and also there was often reporting inconsistency on the inputs used (Table S3).

The most commonly used tool was ResFinder (18 participants), followed by AMRFinderPlus (13 participants). An overview of other tools is available in Figure 6.

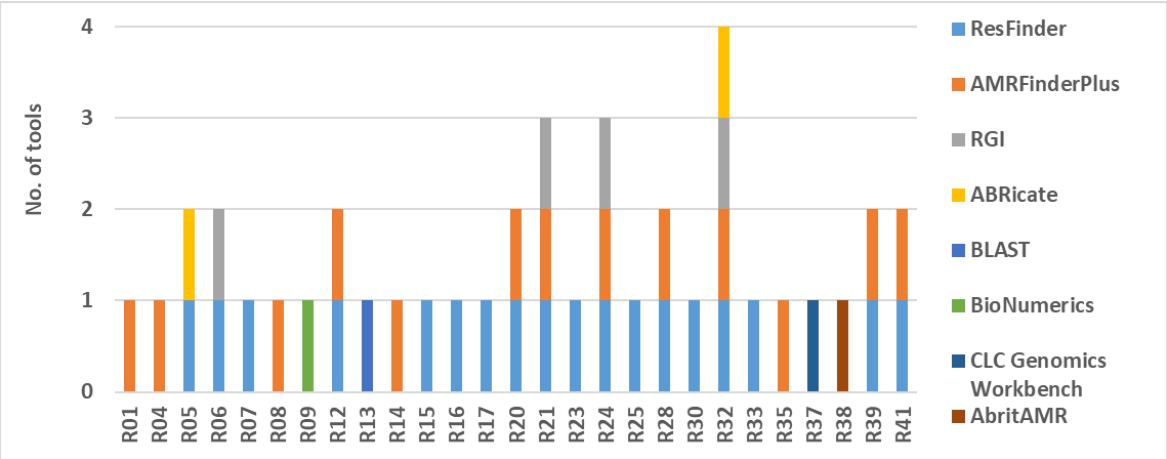

Figure 6. An overview of tools used by 27 participants for AMR gene detection in Campylobacter

The ResFinder database was used by 19 participants and the AMRFinderPlus database was used by 14 participants (Figure 7). The CARD database was used by five participants. The remaining three databases (QMI-AR Nucleotide Database, Generic Acquired Resistance Knowledgebase (GARK) and an in-house database were used by one participant each.

The participants also indicated how they reported AMR genes. Sixteen participants reported all genes detected by the method in use, nine reported a subset of genes based on experience/knowledge/literature and two participants reported a consensus list of genes (common genes present in all databases used) (Figure 7). Additionally, participant R41 indicated that they were avoiding intrinsic and cryptic genes, and participant R32 that reporting was based on 5 tools (Abricate, ResFinder x2, AMRFinder and CARD), where they reported a gene if it was present in 3 tools of 5 with at least 95% identity.

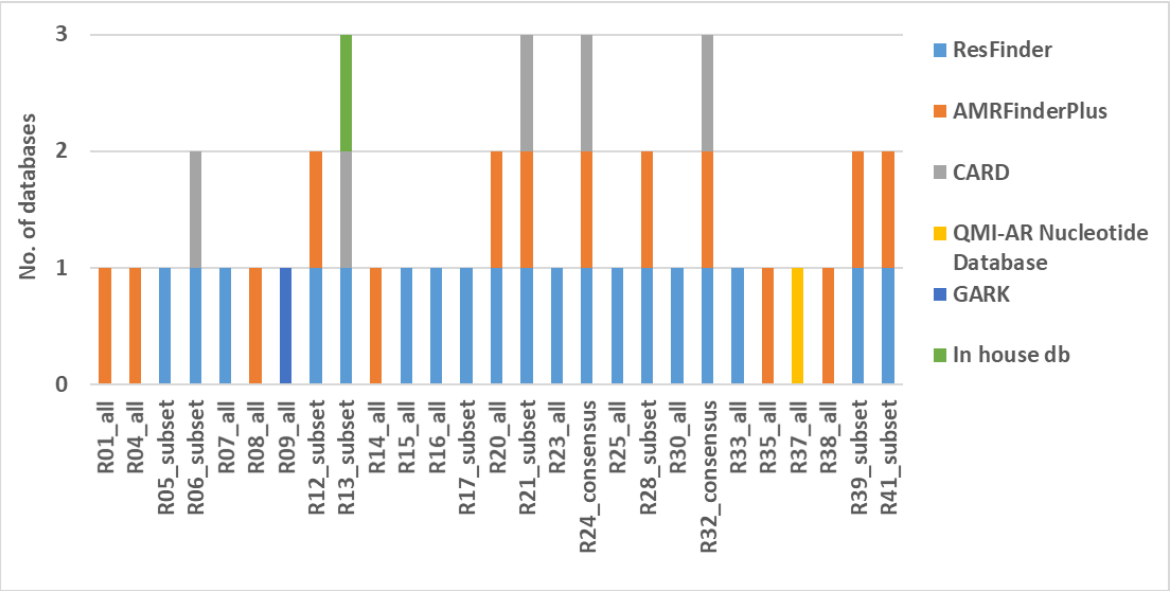

Figure 7. An overview of databases used by 27 participants for AMR gene detection in Campylobacter. The horizontal labels indicate the participant ID and how they reported AMR genes: all – all genes detected by the method in use/from all databases, subset – a subset of genes based on experience/knowledge/literature, consensus – a consensus list of genes (common genes present in all databases used).

4.1.2. Tools and databases used for point mutations detection

All 27 participants were asked to report the tools, the databases, and the inputs that they used for detection of point mutations and also how the point mutations were reported. In addition, they were asked to report the versions of tools and databases used.

Eighteen participants used one tool and one database, five participants - a combination of two tools and two databases, one - one a combination of one tool and two databases, one a combination of two tools and one database, one – a combination of three tools and three databases, and one – a combination of three tools and two databases. Taken all variables (tools/databases/versions/inputs/thresholds/reporting strategies) into account, overall, 26 unique combinations were used by 27 participants. It was observed that participants often were reporting the same version for both tool and the database, and also there was often reporting inconsistency on the inputs used (Table S4).

PointFinder was the preferred tool, being used by 19 participants, either alone or in combination with another tool. AMRFinderPlus was the second most common tool and was used by 12 participants (Figure 8).

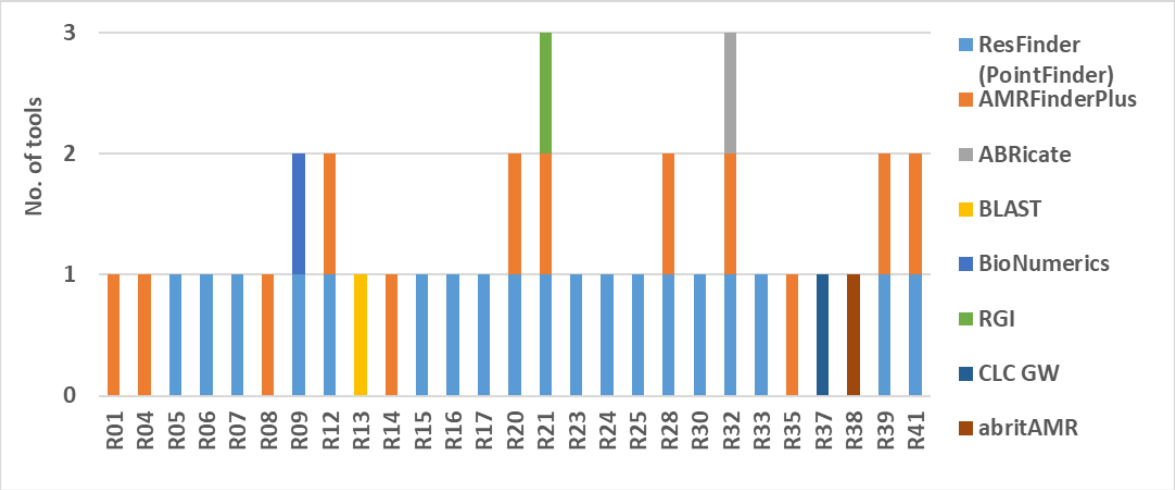

Figure 8. An overview of tools used by 27 participants for point mutations detection in Campylobacter

The ResFinder database was used by 20 participants and the AMRFinderPlus database was used by 13 participants. The CARD, PointFinder in CLC Genomics Workbench and an in-house database were used by one participant each (Figure 9).

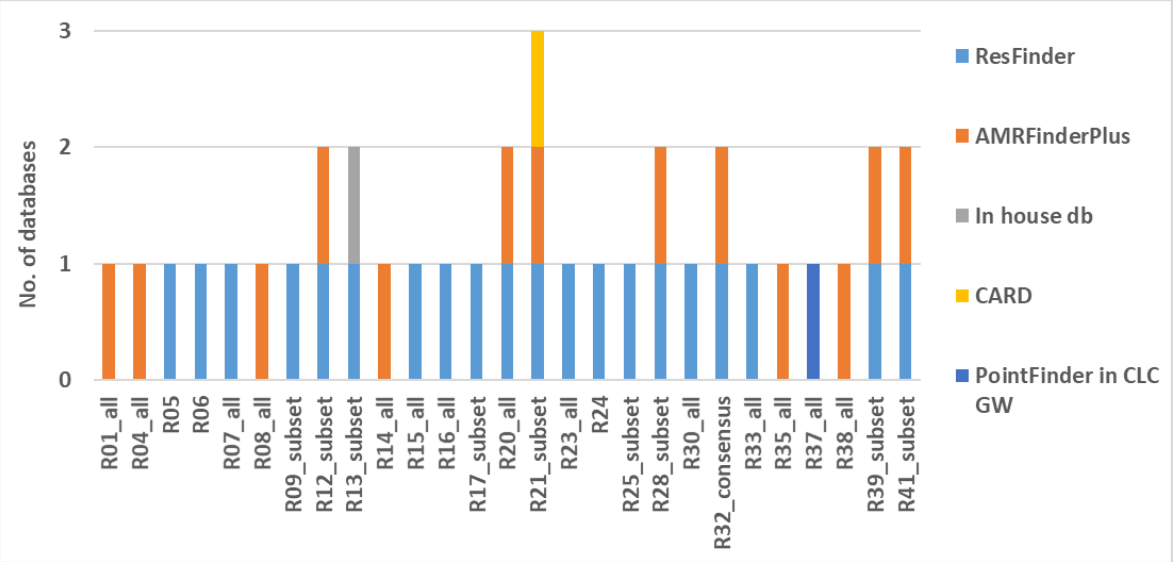

Figure 9. An overview of databases used by 27 participants for point mutations detection in Campylobacter. The horizontal labels indicate the participant ID and how they reported point mutations: all – all point mutations detected by the method in use/from all databases, subset – a subset of point mutations based on experience/knowledge/literature, consensus – a consensus list of point mutations (common point mutations present in all databases used).

4.2. Species and STs reported

4.2.1. Methods used for species identification and results

Twenty-six out of 27 participants reported the species. For sequences RING3C-1 (*C. jejuni*), RING3C-2 (*C. coli*) and RING3C-3 (*C. coli*), all species reported were correct.

The most commonly used tools for species determination were KmerFinder (9 participants) and kraken (6 participants). An overview of the methods used and the reported species is available in Table S7.

4.2.2. Methods used for ST identification and results

Twenty-six out of 27 participants reported the ST for sequence RING3C-1 (ST464), 25 for sequence RING3C-2 (ST12073) and 24 for sequence RING3C-3 (ST828). For sequence RING3C-2, two participants reported an incorrect ST.

MLST (CGE tools) was the most commonly used tool for ST determination (8 participants). For an overview of other methods used, see Table S8.

4.3. AMR genes and PMs reported for Campylobacter isolates

The genes identified by the Ring Trial provider using two different tools and databases, Res_Ref and AMR_Ref, and the genes identified by the participants are presented for each sequence in the following paragraphs.

For each gene and PM table, the concordance of the reported results among the participants was calculated as the percentage of the total number of participants that

reported the same genes or PMs for a given DNA sequence. If a participant deemed the quality of the sequence to be insufficient for reporting genes or PMs for a given sequence, the participant was not included in the calculation for that sequence.

An overview of the observed discrepancies between the two reference datasets in all *Campylobacter* sequences is presented in Table 11 below.

Table 11. Suggested explanation of differences between reference datasets Res_Ref and AMR_Ref, observed in gene and PM reporting for all Campylobacter test sequences. "X" indicates in which database the gene is present.

Gene / PM	Res_Ref	AMR_Ref	Suggested explanation	Reference / comment
50S L22 A103V		X	PM only present in AMRFinderPlus	RING3C-1
aad9		X	*aad9* not present in ResFinder	RING3C-2
aadE		X	Variant in AMRFinderPlus, alternative name: *ant(6)-Ia*	RING3C-2
ant(6)-Ia	X		Variant in ResFinder, alternative name: *aadE*	RING3C-2
aph(2'')-IIa		X	Variant not present in ResFinder	RING3C-3
aph(2'')-Ib	X		Variant not present in AMRFinderPlus	RING3C-3
aph(3')-III	X		Variant not present in AMRFinderPlus	RING3C-3
aph(3')-IIIa		X	Variant present in ResFinder as well, but in RING3C-3 *aph(3')-III* was detected with 100% identity and coverage	RING3C-3
blaOXA		X	In RING3C-1, this variant was the only hit in AMRFinderPlus, at 100% identity and 61% coverage	RING3C-1
blaOXA-193	X		In RING3C-1, gene variant detected with 94% identity and coverage	RING3C-1
catA		X	Gene variant detected with 100% identity and coverage in AMRFinderPlus. Three *catA* variants exist in ResFinder, but in RING3C-2, the best match was *cat(pC194)*	RING3C-2
cat(pC194)	X		Gene variant absent from AMRFinderPlus database, in ResFinder this variant was detected with 100% identity and coverage	RING3C-2
sat4		X	Gene absent from ResFinder database (no genes from streptothricin antibiotics group present)	RING3C-2
tet(O)		X	Both variants *tet(O)* and *tet(O/32/O)* exist in AMRFinderPlus database, but *tet(O)* was given as the only hit for the three sequences with identity of 93% and coverage of 100%	RING3C-1, RING3C-2, RING3C-3
tet(O/32/O)	X		ResFinder database contains both *tet(O)* and *tet(O/32/O)* variants, but this one was found with almost 100% identity and 100% coverage in these three sequences	RING3C-1, RING3C-2, RING3C-3

4.3.1. Sequence RING3C-1

For sequence RING3C-1, all participants reported the tetracycline resistance gene as three different variants, depending on which database was queried. Reporting of *tet(O/M/O)*, however, was surprising, as this variant was not listed among genes detected in Res_Ref or AMR_Ref.

The *blaOXA* gene, however, was reported by 37% of participants (Table 12). In Res_Ref (using reads), the gene was detected as *blaOXA-193* with 94% identity and coverage, but not detected at all when assemblies were used. Correspondingly, participants R05, R07, R16 and R25 from the green category, who did not report the gene, reported using assemblies for analysis (R07 used both reads and assemblies).

In the blue category, out of 6 participants who used only AMRFinderPlus, only R35 reported the *blaOXA* gene. The cut-offs used by this participant were the same as used in AMR_Ref and this gene was identified with 100% identity and 61% coverage. However, participant R08 applied the same cut-offs and did not report the gene. Participants R01, R04, R14 and R38 did not report the *blaOXA* gene, likely due to the applied coverage cut-offs of 90% or 100%. R38 reported the *blaOXA-61* variant instead (Table S14).

Table 12. AMR genes reported in Campylobacter sequence RING3C-1. Reference datasets, Res_Ref and AMR_Ref, are shaded grey. The letter "X" indicates the detection of a specific gene. 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 the 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 +/- other							
	Res_Ref	R05	R07	R15	R16	R17	R23	R25	R30	R33		AMR_Ref	R01	R04	R08	R14	R35	R38	R12	R20	R28	R39	R41	R06	R13	R21	R24	R32	R09	R37	
ResFinder																															
AMRFinderPlus																															
CARD																															
Other																									*					**	***	% concordance
blaOXA												X					X			X											
blaOXA-193	X			X		X	X		X	X													X		X	X						37
tet(O)								X				X	X	X	X	X	X	X	X	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			
tet(O/M/O)		X	X		X															X			X			X		X				100

* In house db
** Generic Acquired Resistance Knowledgebase: 2023.10.27
*** QMI-AR Nucleotide Database (7.0)

The differences in reporting PMs in RING3C-1 among participants were mostly related to the presence or absence of the PMs in different databases (see Table 11). Participant R05 did not report any mutations in this sequence.

Table 13. Point mutations (PMs) reported in Campylobacter sequence RING3C-1. Reference datasets, Res_Ref and AMR_Ref, are shaded grey. The letter "X" indicates the detection of a specific PM. Participants are grouped based on database(s) used: Green – PointFinder, Blue – AMRFinderPlus with or without PointFinder, Yellow – Other (other database(s), specified below the table). Percentage concordance is based on the 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	R05	R06	R07	R09	R15	R16	R17	R23	R24	R25	R30	R33	R37		AMR_Ref	R01	R04	R08	R14	R35	R38	R12	R20	R28	R32	R39	R41		R13	R21	
PointFinder																																
AMRFinderPlus																																
Other																														*	**	% concordance
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	96
50S L22 A103V																X	X	X	X	X	X	X	X	X	X	X	X	X			X	52

* In house db
** RGI- 6.0.3

4.3.2. Sequence RING3C-2

Out of 8 targets (target being a gene reported in the form of different variants) in sequence RING3C-2, only two were reported by more than 90% of participants: *aadE / ant(6)-Ia* and *erm(B)* (Table 14). Eighty-one percent of participants reported the *blaOXA-193* gene. In Res_Ref, it was detected with 96% identity and coverage and when assemblies were used by the Ring Trial provider, the identity and coverage was almost 100%. Participants R05, R07 and R16, who did not report this gene, used assemblies and/or reads with the default cut-offs for identity (90%) and thresholds (60%), so the gene should have been possible to detect. Participants R05 and R07 reported *blaOXA-61* gene instead of *blaOXA-193* (Table S16), which is one of the genes reported by ResFinder when assemblies are used.

The gene *cat(pC194)* was reported only by 5 out of 9 participants using ResFinder, even though in Res_Ref it was detected with 100% coverage and identity.

Participant R04, using AMRFinderPlus, did not report any tetracycline resistance genes, even though in AMR_Ref, the *tet(O)* gene was detected with 94% identity and 100% coverage and the participant used cut-offs of 90% and 60%, respectively.

Table 14. AMR genes reported in Campylobacter sequence RING3C-2. Reference datasets, Res_Ref and AMR_Ref, are shaded grey. The letter "X" indicates the detection of a specific gene. 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 the 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 +/- other							
	Res_Ref	R05	R07	R15	R16	R17	R23	R25	R30	R33	AMR_Ref	R01	R04	R08	R14	R35	R38	R12	R20	R28	R39	R41	R06	R13	R21	R24	R32	R09	R37	% concordance
ResFinder																														
AMRFinderPlus																														
CARD																														
Other																								*				**	***	
aad9											X	X	X	X	X	X	X	X	X	X	X	X					X	X			48
aadE									X		X	X	X	X	X	X	X	X	X	X	X	X				X	X	X			
ant(6)-Ia	X	X	X	X	X	X	X	X		X									X			X	X					X			93
aph(3')-III	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					74
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				81
catA											X	X	X	X	X	X		X	X	X	X	X	X			X	X				
cat(pC194)	X			X	X			X	X	X									X					X	X			X			78
erm(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			100
sat4											X	X	X	X	X	X	X	X	X	X	X	X		X		X	X		X			56
tet(O)											X	X		X	X	X	X		X	X			X			X			X			
tet(O/32/O)	X	X		X		X	X	X	X	X								X	X			X					X	X			78

* In house db
** Generic Acquired Resistance Knowledgebase: 2023.10.27
*** QMI-AR Nucleotide Database (7.0)

The *gyrA* mutation was reported by 93% of the participants and the *rpsL* PM was reported by 85% (Table 15). Participant R16 reported not finding any mutations.

Table 15. Point mutations (PMs) reported in Campylobacter sequence RING3C-2. Reference datasets, Res_Ref and AMR_Ref, are shaded grey. The letter "X" indicates the detection of a specific PM. Participants are grouped based on database(s) used: Green – PointFinder, Blue – AMRFinderPlus with or without PointFinder, Yellow – Other (other database(s), specified below the table). Percentage concordance is based on the 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	R05	R06	R07	R09	R15	R16	R17	R23	R24	R25	R30	R33	R37	AMR_Ref	R01	R04	R08	R14	R35	R38	R12	R20	R28	R32	R39	R41	R13	R21	% concordance
PointFinder																														
AMRFinderPlus																														
Other																												*	**	
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		93
gyrA_2 p.T86I										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		85

* In house db
** RGI- 6.0.3

4.3.3. Sequence RING3C-3

Out of seven targets in sequence RING3C-3, only three targets were reported by more than 90% of participants. R04 deemed the quality of the sequence to be too low and did not report any genes in this sequence.

The results in the blue category (AMRFinderPlus +/- ResFinder) were in general more uniform among participants than in the green category (ResFinder). Participants R07 and R16 reported only one target each. They both used the provided SPAdes assembly. When using the assembly in ResFinder, the Ring Trial provider detected the genes *aph(2'')-Ib*, *aph(3')-III*, *aac(6')-Im*, several *blaOXA* genes and the *tet(O/32/O)* gene with identity and coverage close to 100%. It is therefore unclear why those participants only reported one target, having used the default cut-off values in ResFinder.

Table 16. AMR genes reported in Campylobacter sequence RING3C-3. Reference datasets, Res_Ref and AMR_Ref, are shaded grey. The letter "X" indicates the detection of a specific gene. 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 the 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 +/- other							
	Res_Ref	R05	R07	R15	R16	R17	R23	R25	R30	R33	AMR_Ref	R01	R04[A]	R08	R14	R35	R38	R12	R20	R28	R39	R41	R06	R13	R21	R24	R32	R09	R37	% concordance
ResFinder																														
AMRFinderPlus																														
CARD																														
Other																								*				**	***	
aac(6')-Im	X	X		X		X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	92
aph(2'')-IIa											X	X		X	X	X	X	X	X	X	X	X			X	X	X		X	54
aph(2'')-Ib	X	X		X		X	X	X	X	X									X				X	X	X			X		46
aph(3')-III	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	92
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		81
sat4											X	X		X	X	X	X	X	X	X	X	X	X	X		X	X		X	58
tet(O)											X	X		X	X	X	X		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		
tet(O/M/O)			X																						X					92

* In house db
** Generic Acquired Resistance Knowledgebase: 2023.10.27
*** QMI-AR Nucleotide Database (7.0)
[A] This participant did not report results for this sequence due to reported low sequence quality

Almost all participants detected the two PMs present in RING3C-3 (Table 17).

Table 17. Point mutations (PMs) reported in Campylobacter sequence RING3C-3. Reference datasets, Res_Ref and AMR_Ref, are shaded grey. The letter "X" indicates the detection of a specific PM. Participants are grouped based on database(s) used: Green – PointFinder, Blue – AMRFinderPlus with or without PointFinder, Yellow – Other (other database(s), specified below the table). Percentage concordance is based on the 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	R05	R06	R07	R09	R15	R16	R17	R23	R24	R25	R30	R33	R37	AMR_Ref	R01	R04[A]	R08	R14	R35	R38	R12	R20	R28	R32	R39	R41	R13	R21	% concordance
PointFinder																														
AMRFinderPlus																														
Other																												*	**	
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	
gyrA_2 p.T86I										X			X																	93
23S A2075G	X		X	X	X	X	X	X	X	X	X	X	X	X	X			X	X	X	X	X	X	X	X	X	X	X	X	89

* In house db
** RGI- 6.0.3
* In house db
** Generic Acquired Resistance Knowledgebase: 2023.10.27
*** QMI-AR Nucleotide Database (7.0)
[A] This participant did not report results for this sequence due to reported low sequence quality

5. Conclusions

The RingTrial3 is the third and final Ring Trial exercise in the FWD AMR-RefLabCap project. Sequences from four *Salmonella* and three *Campylobacter* isolates were included.

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.

Participants were free to choose which methods and reporting approaches they used. In this way, they could evaluate whether their routinely used pipeline generated results that were comparable with other Public Health laboratories.

For both *Salmonella* and *Campylobacter*, 26 unique combinations of tools, databases, versions, inputs, thresholds and gene reporting strategies were used by 27 participants.

Many differences in reporting were due to different nomenclature or gene / PM availability in the databases. Despite different nomenclatures, the results could still be compared, considering the alternative names of some genes.

Based on the information about methods, provided by the participants, it was often possible to identify which parameters affected the identification of genes. Too high cut-off values for coverage (both *Salmonella* and *Campylobacter*) or using only assemblies (*Campylobacter*) were the two most common issues.

It needs to be noted that a direct comparison with one of the two reference datasets was only possible for the participants that used one tool: either ResFinder or AMRFinderPlus. For participants that used more than one tool, it was impossible to identify which factor(s) affected diverging reporting.

For this reason, for any next rounds of similar exercises, we would recommend to provide participants with individual reports that would allow more personalized feedback from the Ring Trial provider. This would also open up space for a dialogue between the participant and the ring trial provider or other experts and allow identification of the reasons for missing AMR determinants.

The concordance of the reported AMR determinants among participants and ring trial provider was higher for *Salmonella* than for *Campylobacter* sequences.

An important conclusion of the ring trial was that majority of participants correctly identified the relevant genes and PMs.

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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, versions, inputs, thresholds for sequence coverage and identity used by 27 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 versions, inputs, identity, coverage and same strategy of reporting.

Unique combination	Lab ID[A]	Tool_version_input[B]	Database_version	Identity (%)	Coverage (%)
		1 tool	**1 database**		
1	R33	ResFinder_4.5.0_R	ResFinder_4.5.0	90	90
2	R07[C]	ResFinder_4.5.0_R	ResFinder_4.5.0	90	60
2	R30	ResFinder_4.5.0_R	ResFinder_4.5.0	90	60
3	R17	ResFinder_4.5.0_R	ResFinder_4.5.0	90	60
4	R15	ResFinder_4.5.0_N_R	ResFinder_2.1.1	90	60
5	R23	ResFinder_4.5.0_R	ResFinder_2.2.1	80	60
6	R25	ResFinder_4.5.0_N	ResFinder_2.3.1	90	60
7	R31[D]	ResFinder_4.4.2_N_P_R	ResFinder_4.4.2	90	60
8	R01	AMRFinderPlus_3.11.2_N	AMRFinderPlus_2022-12-19.1	90	100
9	R14	AMRFinderPlus_3.11.26_N	AMRFinderPlus_2023-11-15.1	90	90
10	R08[E]	AMRFinderPlus_3.11.26_N_R	AMRFinderPlus_2023-11-15.1	>90	>50
11	R35[F]	AMRFinderPlus_3.11.11_N	AMRFinderPlus_3.11.11	90	50
12	R42[G]	AMRFinderPlus_3.12.8_N	AMRFinderPlus_2024-01-31.1	90	50
13	R38[H]	AbriTAMR_1.0.15_assemblies	AMRFinderPlus_3.12.8	90	100
		2 tools	**1 database**		
14	R05	ResFinder_4.5_N/ ABRicate_1.0.1_N	ResFinder_4.5	90	60
		2 tools	**2 databases**		
15	R28	ResFinder_4.5.0_N/ AMRFinderPlus_3.12.8_N	ResFinder_2.3.1/ AMRFinderPlus_2024-05-02.2	90/ 90	60/ 50
16	R40[I]	ResFinder_ND_ND/ AMRFinderPlus_3.11.26_R	ResFinder_2.3.1/ AMRFinderPlus_2023-11-15.1	99	99
17	R20	ResFinder_4.5.0_N/ AMRFinderPlus_3.12.8_N	ResFinder_2.3.2/ AMRFinderPlus_2023-11-15	80	60
18	R41[J]	ResFinder_4.5.0_N_R/ AMRFinderPlus_3.12.8_N	ResFinder_2.3.2/ AMRFinderPlus_2024-05-02.2	80	60
19	R39	ResFinder_4.4.2_N/ AMRFinderPlus_3.12.8_N	ResFinder_2.2.1/ AMRFinderPlus_2024-05-02.2	90/ 90	60/ 50
20	R02	ResFinder_4.4.2_N_R/ AMRFinderPlus_3.11.2_N	ResFinder_2.2.1/ AMRFinderPlus_2022-12-19.1	99.5	100
21	R10[K]	ResFinder_4.4.2_N/ AbriTAMR_1.0.14_fasta	ResFinder_2.3.1/ AMRFinderPlus_2024-01-31.1	default	90
22	R06	ResFinder_4.5.0_N_R/ RGI_6.0.3_N	ResFinder_2.3.1/ CARD_3.2.9	90	60
		3 tools	**3 databases**		
23	R21	ResFinder_4.5.0_R/ AMRFinderPlus_3.11.17_N/ RGI_6.0.3_N	ResFinder_'2024-03-22/ AMRFinderPlus_2023-07-13.2/ CARD_3.2.7	90	60
24	R24[L]	ResFinder_4.1.11_N_R/ AMRFinderPlus_3.10.42_N/ RGI_5.2.1_N	ResFinder_2023-03-29/ AMRFinderPlus_2022-10-11.2/ CARD_3.1.4	default for all	default for all
25	R04	AMRFinderPlus_3.12.8_N/ ARIBA_2.14.1_R/ Bionumerics 8.1.1, Salmonella plugin 1.2	AMRFinderPlus_2024-05-02.2/ ResFinder_'2024-05-12/ Resistance KB_'2021-04-12	90/ 90/ 85	90/ 90/ 85
		5 tools	**4 databases**		

26	R32[M]	ResFinder_4.5.0_R/ ResFinder_4.4.2 (EFSA)_R/ AMRFinderPlus_3.11.26_N/ RGI_6.0.3_N/ ABRicate_1.0.1_N	ResFinder_4.5.0/ ResFinder_4.4.2 (EFSA)/ AMRFinderPlus_3.11.26/ CARD_3.2.9/	95	98

[A]In the partcipant ID column: light yellow indicated that theses participants reported all genes detected by the method in use/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).
[B]Inputs: N - DNA fasta, P - protein fasta, R - raw reads
[C]Participant reported that both reads and assemblies were used as input file(s) for identification of AMR genes, however under ResFinder tool reported that only reads were used
[D]Participant reported that protein fasta file was used as an input file to ResFinder. ResFinder tool has no option to use protein fasta for searching AMR genes.
[E]Participant reported that AMRFinderPlus was run in SeqSphere with both fastq and fasta as input files. AMRFinderPlus does not use reads for searching AMR genes. Participant also commented that in our routine we are using >90% ID and 100% coverage, however from experience from RingTrial2, we have chosen to use >90% ID and coverage >50% for RingTrial3.
[F]Participant reported that only reads were used as input file(s) for identification of AMR genes, however under AMRFinderPlus tool reported that assemblies were also used. It also commented that raw reads are input files, then an assembly is created with Spades and analyzed using AMRFinderPlus. This is an in-house pipeline.
[G]Participant reported that both reads and assemblies were used as input file(s) for identification of AMR genes, however under AMRFinderPlus tool reported that only assemblies were used.
[H]Participant did not specify wheather nucleotide or protein fasta file where use as an input to AbriTAMR tool
[I]Participant commented that it reported all genes found with AMRFinder, supplemented with CGE where ID <99%.
[J]when reporting, avoiding intrinsic or cryptic genes, except e.g. OXA-61 in Campylobacter. Participant commented that defaults for both tools were used. However, reported thresholds do not correspond to default settings of the tools used.
[K]Participant reported that only reads were used as input file(s) for identification of AMR genes, however under tools reported that either nucleotide fasta or just fasta were used. Under thresholds, participant commented that for partial matches, 50-90% coverage & >90% identity are used.
[L]Participant reported that only reads were used as input file(s) for identification of AMR genes, however under tools reported that also nucleotide fasta was used
[M]Participant commented: based on our 5 tools (Abricate, ResFinder x2, AMRFinder and CARD), we reporting a gene if it presents in 3 tools on 5 with at least 95% identification

Table S 2. An overview of tools, databases, versions and inputs used by 27 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, versions and same strategy of reporting.

Unique combination	Lab ID[A]	Tool_version_input[B]	Database_version
		1 tool	**1 database**
1	R05	PointFinder_4.5_N	ResFinder_4.5
2	R06	PointFinder_4.5.0_N_R	ResFinder_4.1.0
3	R25	PointFinder_4.5.0_N	ResFinder_4.1.0
4	R07[C]	PointFinder_4.5.0_R	ResFinder_4.5.0
5	R17	PointFinder_4.5.0_R	ResFinder_4.5.0
6	R30	PointFinder_4.5.0_R	ResFinder_4.5.0
6	R33	PointFinder_4.5.0_R	ResFinder_4.5.0
7	R15	PointFinder_4.5.0_N_R	ResFinder_4.0.0
8	R23	PointFinder_4.5.0_R	ResFinder_2.2.1
9	R24[D]	PointFinder_May_2022_N_R	ResFinder_4.1.11
10	R31	PointFinder_4.4.2_N_P_R	ResFinder_4.4.2
11	R01	AMRFinderPlus_3.11.2_N	AMRFinderPlus_2022-12-19.1
12	R08[E]	AMRFinderPlus_3.11.26_N_R	AMRFinderPlus_2023-11-15.1
13	R40	AMRFinderPlus_3.11.26_R	AMRFinderPlus_2023-11-15.1
14	R14	AMRFinderPlus_3.11.26_N	AMRFinderPlus_2023-11-15.1
15	R35[F]	AMRFinderPlus_3.11.11_N	AMRFinderPlus_3.11.11
16	R42[G]	AMRFinderPlus_3.12.8_N	AMRFinderPlus_2024-01-31.1
17	R38[H]	AbritAMR_1.0.15_assemblies	AMRFinderPlus_3.12.8
		2 tools	**2 databases**
18	R02	PointFinder_4.4.2_N_R/ AMRFinderPlus_3.11.2_N	ResFinder_4.0.1/ AMRFinderPlus_2022-12-19.1
19	R20	PointFinder_4.5.0_N/ AMRFinderPlus_3.11.18_N	ResFinder_2.3.2/ AMRFinderPlus_15.1
20	R28	PointFinder_4.5.0_N/ AMRFinderPlus_3.12.8_N	ResFinder_4.1.0/ AMRFinderPlus_2024-05-02.2
21	R39	PointFinder_4.4.2_N/ AMRFinderPlus_3.12.8_N	ResFinder_2.2.1/ AMRFinderPlus_2024-05-02.2
22	R41	PointFinder_4.5.0_N_R/ AMRFinderPlus_3.12.8_N	ResFinder_4.1.0/ AMRFinderPlus_2024-05-2.2

23	R10[I]	PointFinder_4.4.2_N/ AbritAMR_1.0.14_ND	ResFinder_4.1.0/ AMRFinderPlus_2024-01-31.1
24	R04	AMRFinderPlus_3.12.8_N/ Bionumerics 8.1.1_Salmonella plugin 1.2_ND	AMRFinderPlus_2024-05-02.2/ Salmonella Resistance KB_2021.04.12
		3 tools	**3 databases**
25	R21	PointFinder_'2024-03-08_R/ AMRFinderPlus_3.11.17_N/ RGI_6.0.3_ND	ResFinder_'2024-03-08/ AMRFinderPlus_2023-07-13.2/ RGI (CARD)_6.0.3[J]
		4 tools	**3 databases**
26	R32	PointFinder_4.5.0_R/ PointFinder_4.4.2 (EFSA)_R/ AMRFinderPlus_3.11.26_N/ ABRicate_1.0.1_N	ResFinder_4.5.0/ ResFinder_4.4.2 (EFSA)/ AMRFinderPlus_3.11.26

[A]In the partcipant ID column: light yellow indicated that theses participants reporting all point mutations detected by the method in use/from all databases, light red - that participants reported a subset of point mutations based on experience/knowledge/literature, light green - that participants reported a consensus list of point mutations (PMs present in all databases used).
[B]Inputs: N - DNA fasta, P - protein fasta, R - raw reads
[C]Participant reported that both reads and assemblies were used as input file(s) for identification of point mutations, however under ResFinder tool reported that only reads were used
[D]Participant reported that only reads were used as input file(s) for identification of point mutations, however under tools reported that also nucleotide fasta was used
[E]Participant reported that AMRFinderPlus was run in SeqSphere v10.(translated DNA search only). AMRFinderPlus does not use reads or translated DNA to search for pont mutations.
[F]Participant reported that only reads were used as input file(s) for identification of point mutations, however under AMRFinderPlus tool reported that assemblies were also used. It also commented that raw reads are input files, then an assembly is created with Spades and analyzed using AMRFinderPlus. This is an in-house pipeline.
[G]Participant reported that both reads and assemblies were used as input file(s) for identification of point mutations, however under AMRFinderPlus tool reported that only assemblies were used.
[H]Participant did not specify wheather nucleotide or protein fasta file where used as an input to AbriTAMR tool
[I]Participant reported that only reads were used as input file(s) for identification of point mutations, however under tools reported that either nucleotide fasta was used or did not indicate the file in use
[J]Participant reported that RGI was used. We added CARD in brackets for correction.

Table S 3. An overview of the tools, databases, versions, inputs, thresholds for sequence coverage and identity used by 27 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 versions, inputs, identity, coverage and same strategy of reporting.

Unique combination	Lab ID[A]	Tool_version_input[B]	Database_version	Identity (%)	Coverage (%)
		1 tool	**1 database**		
1	R07[C]	ResFinder_4.5.0_N	ResFinder_4.5.0	90	60
1	R16	ResFinder_4.5.0_N	ResFinder_4.5.0	90	60
2	R15[D]	ResFinder_4.5.0_N_R	ResFinder_4.5.0	90	60
3	R17	ResFinder_4.5.0_R	ResFinder_4.5.0	90	60
4	R30	ResFinder_4.5.0_R	ResFinder_4.5.0	90	60
5	R25	ResFinder_4.5.0_N	ResFinder_2.3.1	90	60
6	R33	ResFinder_4.0_R	ResFinder_4.0	90	60
7	R23	ResFinder_2.2.1_R	ResFinder_2.2.1	80	60
8	R01	AMRFinderPlus_3.11.2_N	AMRFinderPlus_2022-12-19.1	100	90
9	R04	AMRFinderPlus_3.12.8_N	AMRFinderPlus_2024-05-02.2	90	60
10	R08[E]	AMRFinderPlus_3.11.26_N_R	AMRFinderPlus_2023-11-15.1	90	50
11	R14	AMRFinderPlus_3.11.26_N	AMRFinderPlus_2023-11-15.1	90	90
12	R35[D]	AMRFinderPlus_3.11.11_N	AMRFinderPlus_3.11.11	90	50
13	R38[G]	AbritAMR_1.0.15_assemblies	AMRFinderPlus_3.12.8	90	100
14	R37	CLC GW_3.8_N	QMI AR_7.0	98	60
15	R09[H]	BioNumerics_8.1.1_R	GARK_'2023-10-27	90	80
		1 tool	**3 databases**		
16	R13	Blast_2.15.0+_N_P	ResFinder_ND/ CARD_ND/ in-house	90	80
		2 tools	**1 database**		
17	R05	ResFinder_4.5_N/ ABRicate_ND_N	ResFinder_4.5	90	60
		2 tools	**2 databases**		
18	R12	ResFinder_4.4.2_N_R/ AMRFinderPlus_3.11.2_N	ResFinder_2.2.1/ AMRFinderPlus_2022-12-19.1	99	100

19	R20	ResFinder_4.5.0_N/ AMRFinderPlus_3.11.18_N	ResFinder_2.3.2/ AMRFinderPlus_15.1	80	60
20	R28	ResFinder_4.5.0_N/ AMRFinderPlus_3.12.8_N	ResFinder_2.3.1 /AMRFinderPlus_2024-05-02.2	90/ 90	60/ 50
21	R39	ResFinder_4.4.2_N/ AMRFinderPlus_3.12.8_N	ResFinder_2.2.1/ AMRFinderPlus_2024-05-02.2	90/ 90	60/ 50
22	R41[I]	ResFinder_4.5.0_N_R/ AMRFinderPlus_3.12.8_N	ResFinder_2.3.2/ AMRFinderPlus_2024-05-2.2	80	60
23	R06	ResFinder_4.5.0_N_R/ RGI_6.0.3_N	ResFinder_2.3.1/ CARD_3.2.9	90	60
		3 tools	**3 databases**		
24	R21	ResFinder_4.5.0_R/ AMRFinderPlus_3.11.17_N/ RGI_6.0.3_N	ResFinder_'2024-03-22/ AMRFinderPlus_2023-07-13.2/ CARD_3.2.7	90	60
25	R24[J]	ResFinder_4.1.11_N_R/ AMRFinderPlus_3.10.42_N/ RGI_5.2.1_N	ResFinder_'2023-03-29/ AMRFinderPlus_2022-10-11.2/ CARD_3.1.4	default	default
		5 tools	**4 databases**		
26	R32[K]	ResFinder_4.5.0_R/ ResFinder_4.4.2 (EFSA)_R/ AMRFinderPlus_3.11.26_N/ RGI_6.0.3_N/ ABRicate_1.0.1_N	ResFinder_4.4.5/ ResFinder_4.4.2 (EFSA)/ AMRFinderPlus_3.11.26/ CARD_3.2.9	>95	>98

[A]In the partcipant ID column: light yellow indicated that theses participants reported all genes detected by the method in use/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).
[B]Inputs: N - DNA fasta, P - protein fasta, R - raw reads
[C]Participant reported that both reads and assemblies were used as input file(s) for identification of AMR genes, however under ResFinder tool reported that only nucleotide fasta were used
[D]Participant reported that only reads were used as input file(s) for identification of AMR genes, however under ResFinder tool reported that both reads and assemblies were used.
[E]Participant commented: in our routine we are using ID > 90% and Coverage=100%. However, in this RingTrial we are using the above mentioned tresholds. (This is based on experiences from RingTrial2).
[F]Participant reported that only reads were used as input file(s) for identification of AMR genes, however under AMRFinderPlus tool reported that nucleotide fasta was used.
[G]Participant did not specify wheather nucleotide or protein fasta file where use as an input to AbriTAMR tool
[H]Generic Acquired Resistance Knowledgebase embedded into BioNumerics, in the Resistance detection plugin was used
[I]avoiding intrinsic and cryptic genes. Participant commented that defaults for both tools were used. However, reported thresholds do not correspond to default settings of the tools used.
[J]Participant reported that only reads were used as input file(s) for identification of AMR genes, however under various tools reported that also nucleotide fasta were used.
[K]Participant commented: based on our 5 tools (Abricate, ResFinder x2, AMRFinder and CARD), we reporting a gene if it presents in 3 tools on 5 with at least 95% identification

Table S 4. An overview of tools, databases, versions and inputs used by 27 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 versions, inputs, and same strategy of reporting

Unique combination	Lab ID[A]	Tool_version_input[B]	Database_version
		1 tool	**1 database**
1	R05	PointFinder_4.5_N	ResFinder_4.5
2	R07[C]	PointFinder_4.5.0_N	ResFinder_4.5.0
2	R16	PointFinder_4.5.0_N	ResFinder_4.5.0
3	R17	PointFinder_4.5.0_R	ResFinder_4.5.0
4	R30	PointFinder_4.5.0_R	ResFinder_4.5.0
5	R06	PointFinder_4.5.0_N_R	ResFinder_4.1.0
6	R25	PointFinder_4.5.0_N	ResFinder_4.1.0
7	R15[D]	PointFinder_4.5.0_N_R	ResFinder_4.0.0
8	R23	PointFinder_4.5.0_R	ResFinder_2.2.1
9	R24[E]	PointFinder_4.1.11_N_R	ResFinder_'2022-May
10	R33	PointFinder_4.0_R	ResFinder_4.0
11	R01	AMRFinderPlus_3.11.2_N	AMRFinderPlus_2022-12-19.1
12	R04	AMRFinderPlus_3.12.8_N	AMRFinderPlus_2024-05-02.2
13	R08[F]	AMRFinderPlus_3.11.26_N_R	AMRFinderPlus_2023-11-15.1
14	R14	AMRFinderPlus_3.11.26_N	AMRFinderPlus_2023-11-15.1
15	R35[G]	AMRFinderPlus_3.11.11_N	AMRFinderPlus_3.11.11
16	R38[H]	AbritAMR_1.0.15_assemblies	AMRFinderPlus_3.12.8
17	R37[I]	CLC GW_ND_R	PointFinder in CLC GW_3.0.1

		1 tool	**2 databases**
18	R13	Blast_2.15.0+_N_P	ResFinder_ND/ In-house
		2 tools	**1 database**
19	R09[J]	PointFinder_4.5.0_R/ BioNumerics_8.1.1_ND	ResFinder_4.1.0
		2 tools	**2 databases**
20	R12	PointFinder_4.4.2_N_R/ AMRFinderPlus_3.11.2_N	ResFinder_4.0.1/ AMRFinderPlus_2022-12-19.1
21	R20	PointFinder_4.5.0_N/ AMRFinderPlus_3.11.18_N	PointFinder_4.1.0, ResFinder_2.3.2/ AMRFinderPlus_15.1
22	R28	PointFinder_4.5.0_N/ AMRFinderPlus_3.12.8_N	ResFinder_4.1.0/ AMRFinderPlus_2024-05-02.2
23	R41	PointFinder_4.5.0_N_R/ AMRFinderPlus_3.12.8_N	ResFinder_4.1.0/ AMRFinderPlus_2024-05-08.8
24	R39	PointFinder_ND_N/ AMRFinderPlus_ND_N	ResFinder_2.2.1/ AMRFinderPlus_2024-05-02.2
		3 tools	**3 databases**
25	R21	PointFinder_4.5.0_R/ AMRFinderPlus_3.11.17_N/ RGI_6.0.3_ND	ResFinder_'2024-03-08/ AMRFinderPlus_3.11.17/ RGI (CARD)_6.0.3[K]
		4 tools	**3 databases**
26	R32	PointFinder_4.5.0_R/ PointFinder_4.4.2 (EFSA)_R/ AMRFinderPlus_3.11.26_N/ ABRicate_1.0.1_N	ResFinder_4.5.0/ ResFinder_4.4.2 (EFSA)/ AMRFinderPlus_3.11.26

[A]In the partcipant ID column: light yellow indicated that theses participants reporting all point mutations detected by the method in use/from all databases, light red - that participants reported a subset of point mutations based on experience/knowledge/literature, light green - that participants reported a consensus list of point mutations (PMs present in all databases used), blank - the participants did not indicate reporting strategy

[B]Inputs: N - DNA fasta, P - protein fasta, R - raw reads

[C]Participant reported that both reads and assemblies were used as input file(s) for identification of AMR genes, however under ResFinder tool reported that only nucleotide fasta were used

[D]Participant reported that only reads were used as input file(s) for identification of AMR genes, however under ResFinder tool reported that both reads and assemblies were used.

[E]Participant reported that only reads were used as input file(s) for identification of AMR genes, however under various tools reported that also nucleotide fasta were used.

[F]Participant commented: run in SeqSphere v10, translated DNA search only. Under AMRFinderPlus tool prticipant reported that both fastq and fasta were used as input files. However, AMRFinderPlus does not use reads for searching AMR genes.

[G]Participant reported that only reads were used as input file(s) for identification of AMR genes, however under AMRFinderPlus tool reported that nucleotide fasta was used.

[H]Participant did not specify wheather nucleotide or protein fasta file where use as an input to AbriTAMR tool

[I]Participant commented: CLC Genomics Workbench Microbial genomic module Find Resistance with PointFinder. PointFinder database for Campylobacter (3.0.1) which uses raw reads.

[J]In BioNumerics the sequences of the 23S and gyrA genes were extracted using a similarity based method against a reference sequence. Cut-offs: 80% minimum identity/ 95% min coverage. Point mutations were screened manually (gyrA T86I and 23S A2075G). In cases where the 23S could not be extracted, Resfinder/PointFinder was used.

[K]Participant reported that RGI was used. We added CARD in brackets for correction.

8. Annex B

8.1. Supplementary tables for serotype/species and ST determination

Table S 5. Salmonella serotypes and methods used for serotype identification by the participants

Lab code	SeqSero	SISTR	ST/eBG (EB)	Other	RING3S-1	RING3S-2	RING3S-3	RING3S-4
R01	X				Corvallis or Chailey*	Typhimurium	Kentucky	potential monophasic variant of Typhimurium
R02	X	X			Corvallis	Typhimurium	Kentucky	4,[5],12 : i : -
R04	X		X		Corvallis	Typhimurium	Kentucky	4,5,12:i:-
R05	X				Corvallis	Typhimurium	Kentucky	I 4,[5],12:i:-
R06	X				8:z4,z23:-	4:i:1,2	8:i:z6	4:i:-
R07	X				Corvallis	Typhimurium	Kentucky	potential monophasic variant of Typhimurium
R08	X				Corvallis	Typhimurium	Kentucky	Monophasic S. Typhimurium
R10	X	X			Corvallis	Typhimurium	Kentucky	monophasic Typhimurium (I 4,[5],12:i:-)
R14		X			Corvallis	Typhimurium	Kentucky	I 1,4,[5],12:i:-
R15	X	X			Corvallis	Typhimurium	Kentucky	Typhimurium;Detected a deletion in gene oafA that causes O5- variant of Typhimurium.
R17	X				Corvallis or Chailey	Typhimurium	Kentucky	potential monophasic variant of Typhimurium
R20	X				8:z4,z23:-	4:i:1,2	8:i:z6	4:i:-
R21	X	X			Corvallis	Typhimurium	Kentucky	Typhimurium (4:i:-)
R23	X				Corvallis	Typhimurium	Kentucky	Typhimurium (monophasic)
R24	X	X			Corvallis	Typhimurium	Kentucky	4,5,12:i:-, monophasic Typhimurium
R25	X				Corvallis	Typhimurium	Kentucky	I 4,[5],12:i:-
R28	X				Corvallis	Typhimurium	Kentucky	Monophasic Salmonella thyphimurium
R30	X				Corvallis, O-8, H-z4, z23	Typhimurium, O-4, H-i, H-1,2	Kentucky, O-8, H-i, H-z6	monophasic Typhimurium, O-4, H-i
R31	X				Salmonella Corvalis	Salmonella Typhimurium	Salmonella Kentucky	Monophasic Typhimurium (4,12: i :-)
R32	X	X			Corvalis	Typhimurium	Kentucky	monophasic variant of Typhimurium
R33	X				Corvallis	Typhimurium	Kentucky	monophasic typhimurium
R35	X			X	S. Corvallis	S. Typhimurium	S. Kentucky	monophasic S. Typhimurium
R38	X				Corvallis	Typhimurium	Kentucky	I 4,[5],12:i:-
R39	X				Corvallis	Typhimurium	Kentucky	Monophasic Typhimurium
R40		X			Corvallis	Typhimurium	Kentucky	1,4,[5],12:-i (Previously described as Monophasic Typhimurium)
R41	X				O-8:z4,z23:-	O-4:i:1,2	O-8:i:z6	O-4:i:-
R42	X	X			Corvallis	Typhimurium	Kentucky	I 1,4,[5],12:i:-

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

Lab code	MLST method	RING3S-1	RING3S-2	RING3S-3	RING3S-4
R01	SeqSphere	1541	19	198	34
R02	SeqSphere	1541	19	198	34
R04	Inhouse pipeline - Bifrost (https://github.com/ssi-dk/bifrost)	1541	19	198	34
R05	MLST2.0 (CGE tools)	1541	19	198	34
R06	MLST2.0 (CGE tools)	1541	19	198	34
R07	Enterobase	1541	19	198	34
R08	SeqSphere	1541	19	198	34
R10	Enterobase	1541	19	198	34
R14	SeqSphere	1541	19	198	34
R15	MLST (tsemann)	1541	19	198	34
R17	MLST2.0 (CGE tools)	1541	19	198	34
R20	MLST (tsemann)	1541	19	198	34
R21	MLST2.0 (CGE tools)	1541	19	198	34
R23	MLST2.0 (CGE tools)	1541	19	198	34
R24	MLST (tsemann)	1541	19	198	34
R25	MLST (tsemann)	1541	19	198	34
R28	MLST (tsemann)	1541	19	198	34
R30	MLST2.0 (CGE tools)	1541	19	198	34
R31	MLST2.0 (CGE tools)	1541	19	198	34
R32	SeqSphere	1541	19	198	34
R33	SRST2	1541	19	198	34
R35	Achtman MLST scheme from Enterobase using in-house pipeline	1541	19	198	34
R38	MLST2.0 (CGE tools)	1541	19	198	34
R39	MLST (tsemann)	1541	19	198	34
R40	SeqSphere	1541	19	198	34
R41	MLST2.0 (CGE tools)	1541	19	198	34
R42	MLST (tsemann)	1541	19	198	34

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

Lab code	KmerFinder	Blast	Kraken	SeqSphere+ Mash Distance	Other	RING3C-1	RING3C-2	RING3C-3
R01				X		C. jejuni	C. coli	C. coli
R04					Kraken/braken part of Bifrost	C. jejuni	C. coli	C. coli
R05			X			C. jejuni	C. coli	C. coli
R06	X					C. jejuni	C. coli	C. coli
R07	X					C. jejuni	C. coli	C. coli
R08				X		C. jejuni	C. coli	C. coli
R09					PubMLST species ID	C. jejuni	C. coli	C. coli
R12				X		C. jejuni	C. coli	C. coli
R13					FastANI	C. jejuni	C. coli	C. coli
R14				X		C. jejuni	C. coli	C. coli
R15					mash with python script	C. jejuni	C. coli	C. coli
R16					/	/	/	/
R17	X					C. jejuni	C. coli	C. coli
R20	X					C. jejuni	C. coli	C. coli
R21	X					C. jejuni	C. coli	C. coli
R23			X			C. jejuni	C. coli	C. coli
R24			X			C. jejuni	C. coli	C. coli
R25	X					C. jejuni	C. coli	C. coli
R28					rMLST	C. jejuni	C. coli	C. coli
R30	X					C. jejuni	C. coli	C. coli
R32				X	PubMLST	C. jejuni	C. coli	C. coli
R33			X			C. jejuni	C. coli	C. coli
R35		X				C. jejuni	C. coli	C. coli
R37					CLC, Find Best Matches using K-mer Spectra.	C. jejuni	C. coli	C. coli
R38			X			C. jejuni	C. coli	C. coli
R39	X		X			C. jejuni	C. coli	C. coli
R41	X					C. jejuni	C. coli	C. coli

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

Lab code	Methods	Other methods	RING3C-1	RING3C-2	RING3C-3
R01	SeqSphere		464	12073	828
R04		Done in Bifrost	464	12073	828
R05	MLST2.0 (CGE tools)		464	12073	828
R06	MLST2.0 (CGE tools)		464		
R07	PubMLST		464	12073	828
R08	SeqSphere		464	12073	828
R09	BioNumerics		464	12073	828
R12	SeqSphere		464	12073	828
R13	PubMLST		464	12073	828
R14	SeqSphere		464	12073	828
R15	MLST (tsemann)		464	12073	828
R16		/			
R17	MLST2.0 (CGE tools)		464	12073	828
R20	MLST (tsemann)		464	12073	828
R21	MLST2.0 (CGE tools)		464	12073	828
R23	MLST2.0 (CGE tools)		464	830	828
R24	MLST (tsemann)		464	12073	828
R25	MLST (tsemann)		464	12073	828
R28	PubMLST		464	12073	828
R30	MLST2.0 (CGE tools)		464	12073	828
R32	SeqSphere		464	12073	828
R33		stringMLST v0.6.3	464	830	828
R35		In-house pipeline using database from PubMLST	464	12073	828
R37	CLC Genomic Workbench		464	12073	
R38	MLST2.0 (CGE tools)		464	12073	828
R39	MLST (tsemann)		464	12073	828
R41	MLST2.0 (CGE tools)		646	12073	828

9. Annex C

Table S 9. Additional genes reported in Salmonella sequence RING3S-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	R05	R07	R15	R17	R23	R25	R30	R31	R33	AMR_Ref	R01	R08	R14	R35	R38	R42	R02	R10	R20	R28	R39	R40	R41	R04	R06	R21	R24	R32
ResFinder																													
AMRFinderPlus																													
CARD																													
Other																									*				
aph(2'')-Ib												X																	
mdsB																				X									
msdB																X													
mdsA*,mdsB* (efflux)																			X										
mdsA																X				X									

* Bionumerics, Salmonella Resistance KB, version 2021.04.12

Table S 10. Additional genes reported in Salmonella sequence RING3S-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	R05	R07	R15	R17	R23	R25	R30	R31	R33	AMR_Ref	R01	R08	R14	R35	R38	R42	R02	R10	R20	R28	R39	R40	R41	R04	R06	R21	R24	R32
ResFinder																													
AMRFinderPlus																													
CARD																													
Other																									*				
aadA2b				X			X																						
qnrS1			X																X										
sul2																				X									
mcr-9.1													X																
mdsA,mdsB (efflux)																			X										
mdsB																X				X									
mcr-9.2							X							X	X					X									
MCR-9.1																							X						
mcr-9						X										X			X						X				
mdsA																X				X									

* Bionumerics, Salmonella Resistance KB, version 2021.04.12

Table S 11. Additional genes reported in Salmonella sequence RING3S-3. 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	R05	R07	R15	R17	R23	R25	R30	R31	R33	AMR_Ref	R01	R08	R14	R35	R38	R42	R02	R10	R20	R28	R39	R40	R41	R04	R06	R21	R24	R32
ResFinder																													
AMRFinderPlus																													
CARD																													
Other																									*				
mdsB																X				X									
mdsA, mdsB																			X										
qacEdelta1																						X						X	
ant(3')-Ia			X																										
mdsA																X				X									

* Bionumerics, Salmonella Resistance KB, version 2021.04.12

Table S 12. Additional PMs reported in Salmonella sequence RING3S-3. 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

	PointFinder												AMRFinderPlus +/- PointFinder													Other	
	Res_Ref	R05	R06	R07	R15	R17	R23	R24	R25	R30	R31	R33	AMR_Ref	R01	R08	R14	R35	R38	R40	R42	R02	R10	R20	R28	R32	R39	R41	R04	R21
PointFinder																													
AMRFinderPlus																													
Other																													
gyrA											X																		
parC											X																		

Table S 13. Additional genes reported in Salmonella sequence RING3S-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	R05	R07	R15	R17	R23	R25	R30	R31	R33	AMR_Ref	R01	R08	R14	R35	R38	R42	R02	R10	R20	R28	R39	R40	R41	R04	R06	R21	R24	R32
ResFinder																													
AMRFinderPlus																													
CARD																													
Other																									*				
mcr-9.1							X						X		X					X									
mdsB																X				X									
mdsA, mdsB																			X										
MCR-9.1																							X						
ant(3'')-Ia																									X				
mcr-9						X													X						X				
mdsA																X				X									
aac(6')-Ib-cr						X																							
aadA2b				X			X																						
ant(6)-Ia			X																										

* Bionumerics, Salmonella Resistance KB, version 2021.04.12

Table S 14. Additional genes reported in Campylobacter sequence RING3C-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 +/- other						
	Res_Ref	R05	R07	R15	R16	R17	R23	R25	R30	R33	AMR_Ref	R01	R04	R08	R14	R35	R38	R12	R20	R28	R39	R41	R06	R13	R21	R24	R32	R09	R37
ResFinder																													
AMRFinderPlus																													
CARD																													
Other																								*				**	***
blaOXA-61																	X			X								X	
cmeB													X							X	X	X							
cmeC																													X

* In house db
** Generic Acquired Resistance Knowledgebase: 2023.10.27
*** QMI-AR Nucleotide Database (7.0)

Table S 15. Additional PMs reported in Campylobacter sequence RING3C-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 databases

	PointFinder														AMRFinderPlus +/- PointFinder												Other	
	Res_Ref	R05	R06	R07	R09	R15	R16	R17	R23	R24	R25	R30	R33	R37	AMR_Ref	R01	R04	R08	R14	R35	R38	R12	R20	R28	R32	R39	R41	R13	R21
PointFinder																												
AMRFinderPlus																												
Other																											*	**
blaOXA-61 G-57T																								X				
23S							X																					

* In house db
** RGI- 6.0.3

Table S 16. Additional genes reported in Campylobacter sequence RING3C-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 +/- other						
	Res_Ref	R05	R07	R15	R16	R17	R23	R25	R30	R33	AMR_Ref	R01	R04	R08	R14	R35	R38	R12	R20	R28	R39	R41	R06	R13	R21	R24	R32	R09	R37
ResFinder																													
AMRFinderPlus																													
CARD																													
Other																								*				**	***
blaOXA-61		X	X	X															X									X	
tet(O/M/O)																									X				
blaOXA-453 to blaOXA-450																			X										
cat(pc194)							X																						
ANT(9)-Ic-aad9																								X					
aph(3')-III (93,33%)									X																				
tet(O-32-O)																								X					
blaOXA-453				X																									
blaOXA-452				X																									
blaOXA-451				X																									
blaOXA-450				X																									
blaOXA-489				X															X										
ANT(6)-Ia																								X					
OXA-605																													X
cat-TC																													X

* In house db
** Generic Acquired Resistance Knowledgebase: 2023.10.27
*** QMI-AR Nucleotide Database (7.0)

Table S 17. Additional genes reported in Campylobacter sequence RING3C-3. 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 +/- other						
	Res_Ref	R05	R07	R15	R16	R17	R23	R25	R30	R33	AMR_Ref	R01	R04	R08	R14	R35	R38	R12	R20	R28	R39	R41	R06	R13	R21	R24	R32	R09	R37
ResFinder																													
AMRFinderPlus																													
CARD																													
Other																								*				**	***
blaOXA-61		X	X	X													X		X		X								
blaOXA-489				X																									
blaOXA-450																													
blaOXA-451																													
blaOXA-452																													
blaOXA-453																													
OXA-605																													X
blaOXA-453 to blaOXA-450																			X										
ant(6)-Ia																			X										
aph(2')-Ib			X																										
blaOXA-489																			X										

* In house db
** Generic Acquired Resistance Knowledgebase: 2023.10.27
*** QMI-AR Nucleotide Database (7.0)

Table S 18. Additional PMs reported in Campylobacter sequence RING3C-3. 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 databases

	PointFinder														AMRFinderPlus +/- PointFinder												Other	
	Res_Ref	R05	R06	R07	R09	R15	R16	R17	R23	R24	R25	R30	R33	R37	AMR_Ref	R01	R04	R08	R14	R35	R38	R12	R20	R28	R32	R39	R41	R13	R21
PointFinder																													
AMRFinderPlus																													
Other																												*	**
OXA promoting region G57T																												X	
blaOXA-61 G-57T																								X					

* In house db
** RGI- 6.0.3

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