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

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Report on the first annual *in vitro* external quality assessment scheme for WGS-based resistome profiling of *Salmonella* and *Campylobacter*

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1. BACKGROUND

This report describes the first External Quality Assessment for WGS-based resistome profiling in antimicrobial-resistant *Salmonella* and *Campylobacter* (EQA1-WGS-AMR), the first out of three planned EQAs, organised by Statens Serum Institut (SSI) in the FWD AMR-RefLabCap project in years 2022-24.

EQA1-WGS-AMR was coordinated with the eighth External Quality Assessment on antimicrobial susceptibility testing (EQA8-AST) of *Salmonella* and *Campylobacter* in the Food- and Waterborne Diseases and Zoonoses Network, which was organised by SSI, as a part of a contract with the European Centre for Disease Prevention and Control (ECDC). EQA8-AST participants, who also participated in EQA1-WGS-AMR, were expected to use the strains received for EQA8-AST in this EQA. All other participants, who participated exclusively in EQA1-WGS-AMR, received packages with the test strains separately.

Thirty-nine participants representing 37 public health laboratories (+ one veterinary institute) from 33 countries were invited. Thirty-one participants accepted the invitation and 25 participants submitted the results. All 31 participants received personal links to the submission form created with the Enalyzer tool (www.enalyzer.com), where they could select to report their results for one or both pathogens.

Participants were encouraged to follow the guidelines in the protocol (https://www.fwdamr-reflabcap.eu/resources/protocols-and-guidelines) developed in the FWD AMR-RefLabCap project.

2. MATERIALS AND METHODS

2.1. Strain selection

Three *Salmonella* and *Campylobacter* strains were selected and represented isolates with different resistance genes and point mutations, as visible in Table 1 and Table 3. The phenotypic resistance profiles of *Salmonella* and *Campylobacter* strains are shown in Table 2 and Table 4, respectively.

Table 1. Characteristics of the *Salmonella* strains selected for the EQA1-WGS-AMR

Strain	Serotype	ST	Genes	Point mutations
EQA_AST.S22.0004	Monophasic Typhimurium	34	*aac(3)-IId, aph(3'')-Ib, aph(6)-Id, blaCTX-M-55, blaTEM-1, floR, mcr-3, qnrS1, sul, tet(A)*	*gyrA* S83Yl
EQA_AST.S22.0005	Heidelberg	15	*aadA, blaCTX-M-123, blaTEM-1 cmlA1, dfrA12, floR, fosA, mph(A) qacL, qnrS1, sul, tet(M)*	None functional
EQA_AST.S22.0008	Senftenberg	14	*aac(3)-II, aac(6')-Ib, aph(3'')-Ib, aph(6)-Id blaCMY-4, blaNDM-1, blaSHV-12, blaTEM-1, ble, qacE, sul1*	*gyrA* D87G *gyrA* S83Y *parC* S80I

Table 2. Phenotypic resistance profiles of the *Salmonella* strains selected for the EQA1-WGS-AMR

Strain	Phenotypic resistance profile[1] (NWT)
EQA_AST.S22.0004	AMP, CAZ, CHL, CIP, CTX, CEP, GEN, NAL, PEF, TCY
EQA_AST.S22.0005	AMP, AZM, CAZ, CHL, CIP, CTX, CEP, PEF, TCY, TMP, TMP-SMX
EQA_AST.S22.0008	AMI, AMP, CAZ, CEP, CIP, CTX, FOX, GEN, MEM, NAL, PEF

[1] Based on MIC data except for PEF and TMP-SMX that are based on DD data.
AMI: Amikacin, AMP: Ampicillin, AZM: Azithromycin, CEP: Cefepime, CTX: Cefotaxime, FOX: Cefoxitin, CAZ: Ceftazidime, CHL: Chloramphenicol, CIP: Ciprofloxacin, COL: Colistin, , GEN: Gentamicin, MEM: Meropenem, NAL: Nalidixic acid, PEF: Pefloxacin, SMX: Sulfamethoxazole, TCY: Tetracycline, TRI: Trimethoprim

Table 3. Characteristics of the *Campylobacter* strains selected for the EQA1-WGS-AMR

Strain	Species	ST	Genes	Point mutations
EQA_AST.C22.0001	*C. jejuni*	7433	*aad9, aph(2'')-If, aph(3')-III, blaOXA-193, cat, tet(O)*	*gyrA* T86I, 50S_L22 A103V
EQA_AST.C22.0004	*C. coli*	872	*aac(6')-aph(2''), aadE, ant(6)-Ia, aph(3')-III, blaOXA-193, sat4, tet(O)*	*gyrA* T86I
EQA_AST.C22.0005	*C. coli*	872	*aadE-Cc, blaOXA-489, tet(O)*	*gyrA* T86I, 23S_A2075G

Table 4. Phenotypic resistance profiles of the *Campylobacter* strains selected for the EQA1-WGS-AMR

Strain	Resistance profile[1]
EQA_AST.C22.0001	Ciprofloxacin, Gentamicin, Tetracycline
EQA_AST.C22.0004	Ciprofloxacin, Gentamicin, Tetracycline
EQA_AST.C22.0005	Ciprofloxacin, Erythromycin, Tetracycline

[1] Based on EUCAST ECOFF MIC values.

2.2. WGS analysis by the EQA provider

A selection of candidate strains was sequenced using Illumina paired-end sequencing. The strains were subcultured 10 times and sequenced again. This step was performed to ensure that the test strains exhibited a stable genotype. The quality of sequences (genome size, N50, and total number of contigs) was checked with an in-house QC pipeline (https://github.com/ssi-dk/bifrost) for raw reads and an open source script (https://github.com/hcdenbakker/N50.sh) for assemblies.

Campylobacter species identification was done using Kraken (https://github.com/DerrickWood/kraken). MLST calling was done with ARIBA (https://github.com/sanger-pathogens/ariba) using the typing schemes from the PubMLST database.
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* Java from *S* Paratyphi B.

The sequences were analysed for antimicrobial resistance genes and point mutations (PMs) to generate 2 reference datasets:

a. FWD01Res: ResFinder (raw reads)
b. FWD01Amr: AMRFinderPlus (SPAdes assemblies)

a) FWD01Res: Antibiotic resistance genes were identified using raw reads mapped with ARIBA to the ResFinder database, then run through ABRicate (https://github.com/tseemann/abricate) with the ResFinder database. Point mutations were identified using KMA and an in-house custom database of point mutations (https://github.com/ssi-dk/punktreskma).
b) FWD01Amr: Antibiotic resistance genes were identified using SPAdes assemblies that were run through AMRFinderPlus. Point mutations were also identified using AMRFinderPlus.

2.3. Enalyzer survey

A reporting scheme was developed in Enalyzer survey tool. Participants received individual links where they could submit their results within 3 months from receiving the strains.

The first part of the survey included questions about the basic quality parameters for each strain, such as genome size, total number of contigs and the N50 number. The second part focused on tools and databases used to identify the ST, AMR genes, point mutations, as well as serotype and species for *Salmonella* and *Campylobacter*, respectively. In the third part, the genes were grouped according to the antimicrobial class.

There, it was possible, reporting one strain at a time, to select the identified genes from a pre-defined list. Furthermore, for each antimicrobial class there was an option of entering additional genes in text boxes. For reporting of point mutations, the participants were asked to type the detected mutations in text boxes as well.

2.4. Data analysis

Most of the laboratories reported results for both pathogens, but some countries submitted either for *Salmonella* or for *Campylobacter*. For this reason, we had a total of 25 participants, of which 21 submitted *Salmonella* data and 20 *Campylobacter* data. The participants were from Austria, Belgium, Bulgaria, Denmark, Finland, France, Germany, Hungary, Ireland, Italy, Latvia, Luxembourg, Malta, Norway, Poland, Portugal, Romania, Serbia, Slovenia, Spain, Sweden and the Netherlands. Participants were assigned random codes FWDXX.

We collected the submissions from all the participants after the deadline and analysed them by comparing the reported genes and point mutations to two reference data sets generated by the EQA provider with different databases and tools, as shown in Table 5.

Table 5. Tools and databases used in provider's reference data sets for *Salmonella* and *Campylobacter*

Reference data set name	Tools and databases applied	
	Gene detection	**Point mutation identification**
FWD01Amr	AMRFinderPlus on SPAdes assemblies	AMRFinderPlus on SPAdes assemblies
FWD01Res	ARIBA/ABRicate with ResFinder database	KMA with in-house developed Point Mutation database*

*Based on PointFinder database

The two reference data sets were generated based on two different databases for AMR gene detection and point mutation identification: ResFinder and AMRFinderPlus. This was in order to be able to compare whether similar tools and databases would generate similar results.

3. SALMONELLA RESULTS

3.1. Quality metrics for all *Salmonella* strains

In order to assess the general quality of the sequences and assemblies produced (if relevant), we asked the participants to report the assembled genome size, the total number of contigs, as well as the N50 value, which represents the shortest contig length at 50% of the total assembly length. In this Quality metrics section, laboratory FWD01 represents the provider's reference data set.

The participants reported very uniform assembled genome sizes of approximately 5 Mb for all three *Salmonella* strains, with the exception of laboratory FWD10, where the genome sizes for all three strains were higher and reaching above 6 Mb for strain EQA_AST.S22.0008 (Figure 1).

Figure 1. The size of assembled genomes of each strain: orange – EQA_AST.S22.0004, yellow – EQA_AST.S22.0005 and green – EQA_AST.S22.0008.

The total number of contigs varied greatly among the participants (Figure 2). The WGS protocol includes recommendations for fewer than 500 contigs (Bortolaia et al., 2020) (excluding contigs <300 bp), however it was not clear whether small contigs were filtered before reporting the number of contigs, as the provider did not ask about filtering. A high number of contigs may indicate a mixed culture.

Figure 2. Total number of contigs in Salmonella strains: orange – EQA_AST.S22.0004, yellow – EQA_AST.S22.0005 and green – EQA_AST.S22.0008.

The N50 value, defined as the shortest contig length at 50% of the total assembly length, also varied between the laboratories. In general, the higher the N50 number, the longer the contigs and therefore, the better an assembly is.

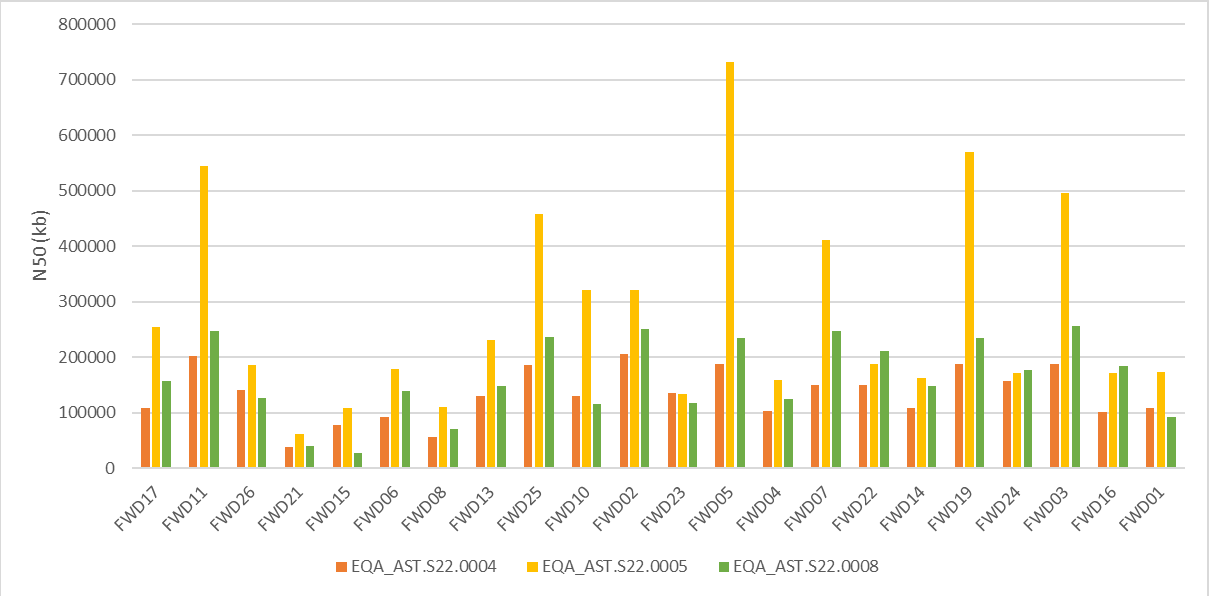

Figure 3. The N50 value for all three strains: orange – EQA_AST.S22.0004, yellow – EQA_AST.S22.0005 and green – EQA_AST.S22.0008.

Overall, the qualities of the sequences and assemblies submitted by the participants were of good quality. There is no golden standard as to minimum requirements for reliable gene detection and point mutation identification, however, some authors suggest, among other parameters, N50 of >30000 bp and 500 as the maximum number of contigs (Bortolaia et al., 2020).

3.2. AMR gene and PMs detection methods used

The methods used by the participants varied, however, the majority of participants used the ResFinder database, either on assemblies or using raw reads. Different tools were used to query or map to the database. The second most used database was AMRFinderPlus, and the input for analysis in AMRFinderPlus was assemblies primarily made with SKESA or SPAdes. SPAdes was used by 8 participants and SKESA was used by 6. Other participants used Velvet, CLC Genomics or Shovill.

It was noted that many participants used SKESA for assemblies. SKESA is a fast assembler which is optimal for assembling genes in e.g. core genome typing schemes. However, it should be noted that the developers of SKESA recommend SAUTE (Souvorov & Agarwala, 2021) for assembling repeat regions and other regions that are difficult to assemble, and therefore also for assembling AMR genes that are often present on plasmids and casettes. We did not, however, see a pattern of fewer AMR genes detected for participants using SKESA with AMRFinderPlus.

Finally, it should be mentioned that some participants reported that they were not sure which tools and databases they used for AMR gene detection and point mutation detection. Figure 4 summarizes different tools reported as used by the participants for antimicrobial resistance gene detection.

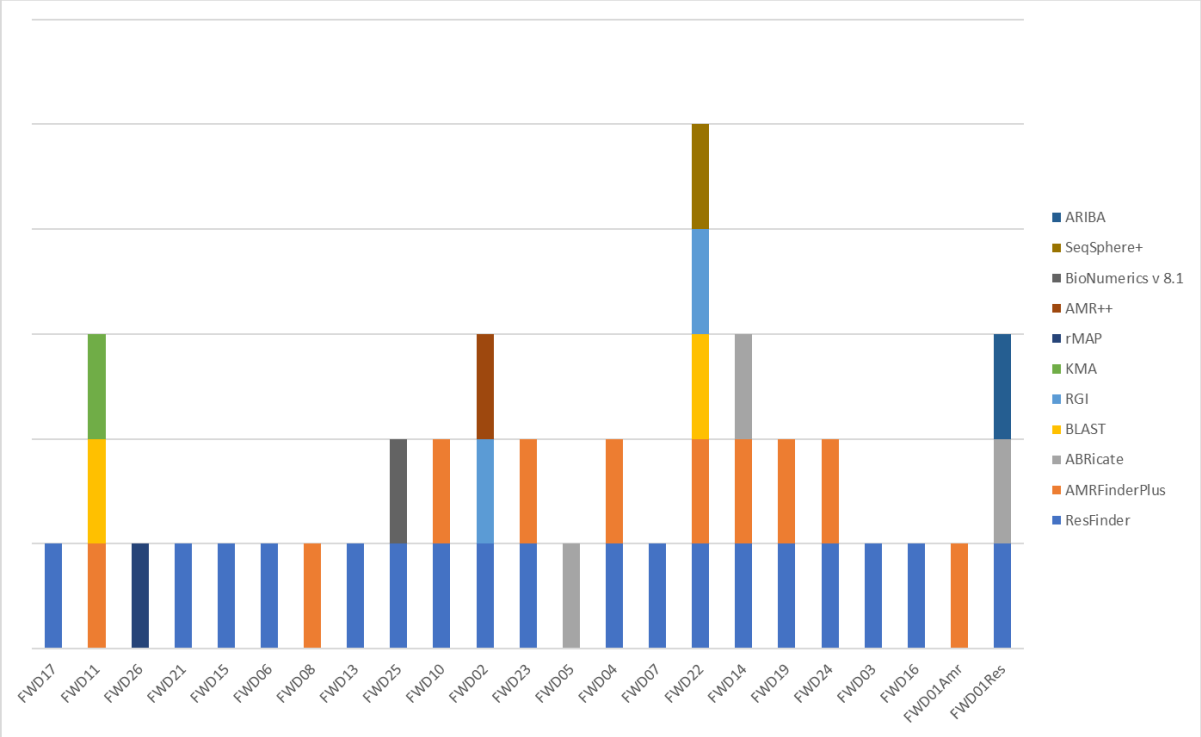

Figure 4. Tools used by the participants for AMR gene detection for all *Salmonella* strains.

Taken together, ResFinder and AMRFinderPlus were the two most commonly used tools. Eight participants used ResFinder alone and nine in combination with other tools. AMRFinderPlus was used by one participating laboratory as the only tool and by eight laboratories in combination with other methods.

The tools used for point mutations detection for all participants are summarized in Figure 5.

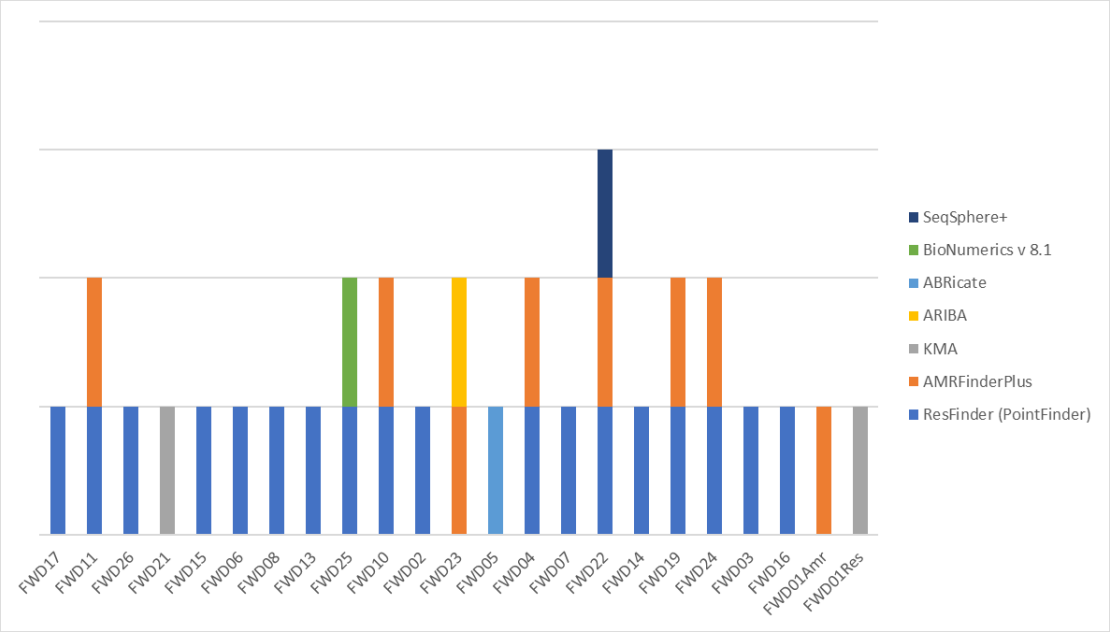

Figure 5. Overview of tools used for point mutation detection by all participants for all *Salmonella* strains.

For point mutation detection, PointFinder was by far the preferred tool, being used by 11 out of 21 laboratories as the only tool and by seven in combination with other tools. AMRFinderPlus was the second most common tool, being used by seven participating laboratories.

3.3. Serotypes and STs reported

Participants were asked to report the serotype and ST of all three *Salmonella* strains. All participants reported the expected ST's on strains EQA_AST.S22.0004 and EQA_AST.S22.0005. For strain EQA_AST.S22.0008 all but one participant reported the correct ST. The latter participant did not report the ST on this strain.
Serotypes were reported as seen in Table 6 and overall correctly predicted.The majority of participants reported strain EQA_AST.S22.0004 correctly as monophasic Typhimurium, however some participants did not differentiate between Typhimurium and the monophasic variant. Two participants did not correctly predict the *S*. Heidelberg and two did not correctly predict the *S*. Senftenberg. Furthermore, three participants were not able to distinguish whether the strain EQA_AST.S22.0008 was *S*. Senftenberg or *S*. Dessau. A look-up of the ST in Enterobase would help differentiate between the two.

Table 6. *Salmonella* serotypes reported by the participants

Lab#	EQA_AST.S22.0004	EQA_AST.S22.0005	EQA_AST.S22.0008
FWD17	Monophasic Typhimurium	Heidelberg	Senftenberg
FWD11	Monophasic Typhimurium	Heidelberg	Senftenberg
FWD26	Monophasic Typhimurium	Heidelberg	Senftenberg
FWD21	Typhimurium	Heidelberg	Senftenberg
FWD15	Monophasic Typhimurium	Heidelberg	Senftenberg
FWD06	Monophasic Typhimurium	Heidelberg	Senftenberg
FWD08	Typhimurium	Heidelberg	Senftenberg
FWD13	Monophasic Typhimurium	Heidelberg	6,7 : g,m,s,t : -
FWD25	Monophasic Typhimurium	Heidelberg	Senftenberg
FWD10	Monophasic Typhimurium	Heidelberg	Senftenberg
FWD02	Typhimurium	Heidelberg	Senftenberg
FWD23	Monophasic Typhimurium	Heidelberg	Senftenberg
FWD05	Typhimurium	Senftenberg	Heidelberg
FWD04	Monophasic Typhimurium	Heidelberg	Senftenberg
FWD07	Monophasic Typhimurium	Heidelberg	Senftenberg
FWD22	Monophasic Typhimurium	Heidelberg	Senftenberg or Dessau
FWD14	Monophasic Typhimurium	Heidelberg	Senftenberg
FWD19	Monophasic Typhimurium	Heidelberg	Senftenberg or Dessau
FWD24	Monophasic Typhimurium	Heidelberg	Senftenberg
FWD03	Monophasic Typhimurium	Heidelberg	Senftenberg
FWD16	Monophasic Typhimurium	4:-:-	Senftenberg or Dessau

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

In general, most participants demonstrated a qualified detection of AMR genes and point mutations in the three *Salmonella* strains used in this EQA. Only a few laboratories had problems in detecting a few genes and point mutations. We suspect that not all participants reported the point mutations and therefore are listed as not detecting any.

The gene *aac(6')-Iaa* was reported by approximately half of the participants. This gene is endogenous to the *Salmonella* genus, however, it is considered a cryptic gene that does not contribute to aminoglycoside resistance (Magnet et al., 1999), hence, it might not be reported in certain databases (Bharat et al., 2022). Reporting of the gene as both present and as absent is considered a qualified answer as we did not ask for a predicted phenotype.

In the following strain-specific tables, the expected antibiotic resistance genes for each strain are marked with an "X" in the columns FWD01Res and FWD01Amr, referring to the two reference datasets as explained in Table 5. The participants are grouped into four categories. The first two categories are based on the two main tools used to identify the antibiotic resistance genes: ResFinder (Green) and AMRFinderPlus (Orange), together with the corresponding reference datasets (FWD01Res and FWD01Amr). Participants that have used more than one tool are grouped in the "Mixed methods" (Yellow) category. The "Other" (Blue) category indicates the participants that reported using a single other tool. This was ABRicate and rMAP version 1.0 for laboratories FWD05 and FWD26, respectively. For an overview of the tools used by each participant for antibiotic gene detection, see Figure 4.

The expected point mutations are also marked with an "X" in separate strain-specific tables in columns FWD01Amr and FWD01Res. The participants are grouped into the same categories as for the antibiotic gene detection. For an overview of which tools different participants used for point mutation detection, see Figure 5.

3.4.1. Strain EQA_AST.S22.0004

Strain EQA_AST.S22.0004 is a monophasic Typhimurium strain, ST 34.

Table 7. Genes found in strain EQA_AST.S22.0004, Green – ResFinder, Red – AMRFinder, Yellow – mixed methods, Blue – single other method different from ResFinder and AMRFinder. Columns with reference results, FWD01Res and FWD01Amr, are highlighted in light grey.

Lab#	FWD01Res	FWD17	FWD21	FWD15	FWD06	FWD13	FWD07	FWD03	FWD16	FWD01Amr	FWD08	FWD11	FWD25	FWD10	FWD02	FWD23	FWD04	FWD22	FWD14	FWD19	FWD24	FWD26	FWD05
	ResFinder									AMRFinder		Mixed methods										Other	
aac(3)-IId	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
aac(6)-Iaa *												X						X					
aac(6')-Iaa		X		X		X	X	X	X						X		X		X				X
aph(3)-Ib *												X											
aph(3')-Ib *																							X
aph(3'')-Ib	X	X	X	X	X	X	X	X	X	X	X			X	X	X		X	X	X	X	X	
aph(3)-Id												X											
aph(6)-Id	X	X	X	X	X	X	X	X	X	X	X			X	X	X		X	X	X	X	X	
aph(6')-Id *																							X
blaCTX-M-55	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
blaTEM-1	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
floR	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
mcr-3	X	X	X	X		X	X	X	X	X		X	X	X	X	X	X	X	X	X	X		X
mdsA																						X	
mdsB																						X	
qnrS1	X	X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X
sul	X	X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X
tet(A)	X	X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X

* – Correct gene, but likely reported with a typo

The same genes were detected by the two different tools in the reference data sets FWD01Res and FWD01Amr. All the participants identified genes *aac*(3)-IId, *bla*CTX-M-55, *bla*TEM-1 and *floR*. The three genes *qnrS1*, *sul* and *tet(A)* were reported by 20 out of 21 participants. For the remaining genes, there was more variation in detection among participants.

There was one expected point mutation in strain EQA_AST.S22.0004, *gyrA* S83Y. This was identified in both reference datasets, FWD01Amr and FWD01Res. Eighteen out of 21 laboratories also identified this mutation (Table 8), irrespective of the tool used. The tools used by the three laboratories that did not report this mutation were PointFinder (FWD02 and FWD08) and ABRIcate (FWD05). This is surprising, since 10 other laboratories did successfully identify this mutation using PointFinder.

Table 8. Point mutation found in strain EQA_AST.S22.0004, Green – ResFinder, Red – AMRFinder, Yellow – mixed methods, Blue – single other method different from ResFinder and AMRFinder. Columns with reference results, FWD01Res and FWD01Amr, are highlighted in light grey.

Lab#	FWD17	FWD26	FWD15	FWD06	FWD13	FWD07	FWD14	FWD03	FWD16	FWD08	FWD02	FWD01Amr	FWD25	FWD11	FWD10	FWD23	FWD04	FWD22	FWD19	FWD24	FWD01Res	FWD21	FWD05
	ResFinder											AF		Mixed methods							Other		
gyrA S83Y	X	X	X	X	X	X	X	X	X			X	X	X	X	X	X	X	X	X	X	X	

For an overview of which tools different participants used, see Figure 5. One laboratory reported point mutations in *E. coli* and *H. influenzae* (not shown), which are not relevant in *Salmonella*.

3.4.2. Strain EQA_AST.S22.0005

Strain EQA_AST.S22.0005 is a Senftenberg, ST14.

Table 9. Genes found in strain EQA_AST.S22.0005, Green – ResFinder, Red – AMRFinder, Yellow – mixed methods, Blue – single other method different from ResFinder and AMRFinder. Columns with reference results, FWD01Res and FWD01Amr, are highlighted in light grey.

Lab#	FWD01Res	FWD17	FWD21	FWD15	FWD06	FWD13	FWD07	FWD03	FWD16	FWD01Amr	FWD08	FWD11	FWD25	FWD10	FWD02	FWD23	FWD04	FWD22	FWD14	FWD19	FWD24	FWD26	FWD05
	ResFinder									AMRFinder		Mixed methods										Other	
aac(6')-Iaa		X		X		X	X	X	X			X			X			X	X				X
aadA	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
blaCTX-M-123	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
blaTEM-1	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
cmlA1	X	X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X
dfrA12	X	X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X
floR	X	X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X
fosA	X	X	X	X			X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X
mph(A)	X		X		X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X	X
qacL										X				X	X								
qnrS1	X	X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X
sul	X	X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X
tet(M)	X	X	X		X	X			X	X		X	X	X	X	X	X	X			X	X	X

For this strain, minor differences were observed between the two reference datasets, FWD01Res and FWD01Amr. The differences are caused by the different databases queried. Gene *qacL* is present in AMRFinderPlus, but not in ResFinder.

Among the participants, there was an unanimous detection of gene *blaCTX-M-123*, as well as *blaTEM-1* gene. Genes *cmlA1*, *dfrA12*, *floR*, *qnrS1* and *sul* were detected by all participants except FWD08. Gene *qacL* was reported only by two laboratories, in addition to the reference dataset generated with AMRFinderPlus. This gene encodes a subunit of a multidrug efflux pump and contributes to disinfectant (quaternary ammonium compound) resistance (Ceccarelli et al., 2006). It is likely that the participants only focused on reporting antibiotic resistance genes and for this reason did not report this gene. Furthermore, as noted above, the gene is only present in the AMRFinderPlus database and not in the ResFinder database.

There was one point mutation in strain EQA_AST.S22.0005, *parC* T57S, that was reported by 13 out of 21 participants, but not reported in any of the reference data sets (Table 10).

Table 10. Point mutation reported in strain EQA_AST.S22.0005, Green – ResFinder, Red – AMRFinder, Yellow – mixed methods, Blue – single other method different from ResFinder and AMRFinder. Columns with reference results, FWD01Res and FWD01Amr, are highlighted in light grey.

Lab#	FWD17	FWD26	FWD15	FWD06	FWD13	FWD07	FWD14	FWD03	FWD16	FWD08	FWD02	FWD01Amr	FWD25	FWD11	FWD10	FWD23	FWD04	FWD22	FWD19	FWD24	FWD01Res	FWD21	FWD05
	ResFinder											AF	Mixed methods								Other		
parC T57S	X		X	X	X	X	X	X	X					X	X		X	X	X				

It was suggested previously that this mutation could be a naturally occurring compensatory mutation (Eaves et al., 2004) and there is currently no consensus whether it contributes to quinolone resistance in *Salmonella* spp. (Chang et al., 2021). The mutation is not present in the AMRFinderPlus database but it is present in the PointFinder database.

3.4.3. Strain EQA_AST.S22.0008

Strain EQA_AST.S22.0008 is a Heidelberg strain, ST14.

Table 11. Genes found in strain EQA_AST.S22.0008, Green – ResFinder, Red – AMRFinder, Yellow – mixed methods, Blue – single other method different from ResFinder and AMRFinder. Columns with reference results, FWD01Res and FWD01Amr, are highlighted in light grey.

Lab#	FWD01Res	FWD17	FWD21	FWD15	FWD06	FWD13	FWD07	FWD03	FWD16	FWD01Amr	FWD08	FWD11	FWD25	FWD10	FWD02	FWD23	FWD04	FWD22	FWD14	FWD19	FWD24	FWD26	FWD05
	ResFinder									AMRFinder		Mixed methods										Other	
aac(3)-II	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
aac(6')-Ib		X		X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
aac(6')-Ib-cr		X		X	X	X	X	X	X						X				X		X		
aac(6')-Iaa		X		X		X	X	X	X			X			X			X	X				X
aph(3')-Ib *												X											
aph(3'')-Ib	X	X	X	X	X	X	X	X	X	X	X			X	X			X	X	X	X	X	X
aph(6)-Id	X	X	X	X	X	X	X	X	X	X	X			X	X			X	X	X	X	X	
aph(6')-Id *												X											X
blaCMY-4	X	X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X
blaNDM-1	X	X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X
blaSHV-12	X	X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X
blaTEM-1	X	X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X
ble										X		X		X				X				X	
mdsA																						X	
mdsB																						X	
qacE	X		X					X	X	X		X		X	X								
qnrB19																				X			
sul1	X	X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X

* – Correct gene, but likely reported with a typo

In this strain, minor differences between the two reference datasets, FWD01Res and FWD01Amr, were observed. Gene *aac(6')-Ib* and *ble* were detected by AMRFinderPlus, but not by ResFinder. All laboratories, apart from FWD08, reported the presence of *sul1*, *blaCMY-4*, *blaNDM-1*, *blaSHV-12* and *blaTEM-1* genes.

Three functional point mutations were expected in strain EQA_AST.S22.0008 (Table 12) and they were identified by both reference datasets, FWD01Amr and FWD01Res, as well as by the majority of participants.

Table 12. Point mutation reported in strain EQA_AST.S22.0008, Green – ResFinder, Red – AMRFinder, Yellow – mixed methods, Blue – single other method different from ResFinder and AMRFinder. Columns with reference results, FWD01Res and FWD01Amr, are highlighted in light grey.

Lab#	FWD17	FWD26	FWD15	FWD06	FWD13	FWD07	FWD14	FWD03	FWD16	FWD08	FWD02	FWD01Amr	FWD25	FWD11	FWD10	FWD23	FWD04	FWD22	FWD19	FWD24	FWD01Res	FWD21	FWD05
	ResFinder											AF	Mixed methods								Other		
gyrA D87G	X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	
gyrA S83Y	X	X	X	X	X	X	X	X	X			X	X	X	X	X	X	X	X	X	X	X	
parC S80I	X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	
parC T57S	X		X	X	X	X	X	X	X				X	X			X	X	X				

One laboratory reported other mutations known from *E. coli* and *H. influenzae* (not shown), that are not relevant in *Salmonella*. The *parC* T57S substitution, classified as non-informative by AMRFinderPlus was reported by the same laboratories as for the strain EQA_AST.S22.0005.

4. CAMPYLOBACTER RESULTS

4.1. Quality metrics for all *Campylobacter* strains

In order to assess the general quality of the sequences and assemblies produced, we asked the participants to report the assembled genome size, total number of contigs, as well as the N50 value, which represents the shortest contig length at 50% of the total assembly length. In this section, laboratory FWD01 represents the provider's reference data set. The reported genome sizes for all laboratories for all the strains were uniform and below 1.8 MB.

Figure 6. The size of assembled genomes of each strain: orange – EQA_AST.C22.0001, yellow – EQA_AST.C22.0004 and green – EQA_AST.C22.0005. No data available for strain EQA_AST.C22.0005 for laboratory FWD20 due to failing of sequencing.

There was little variation in the total number of contigs among the participants (Figure 7). The provider's laboratory, FWD01, reported one of the highest number of contigs, reaching 300 for strain EQA_AST.C22.0005. Additionally, one laboratory reported a contig number above 300 for the same strain. The WGS protocol includes recommendations for fewer than 500 contigs in general (Bortolaia et al., 2020) (excluding contigs <300 bp). *Campylobacter* genomes can be assembled typically into less than 100 contigs. However, it was not clear whether in this EQA small contigs were filtered before reporting the number of contigs, as the provider did not ask about filtering. A high number of contigs may indicate a mixed culture.

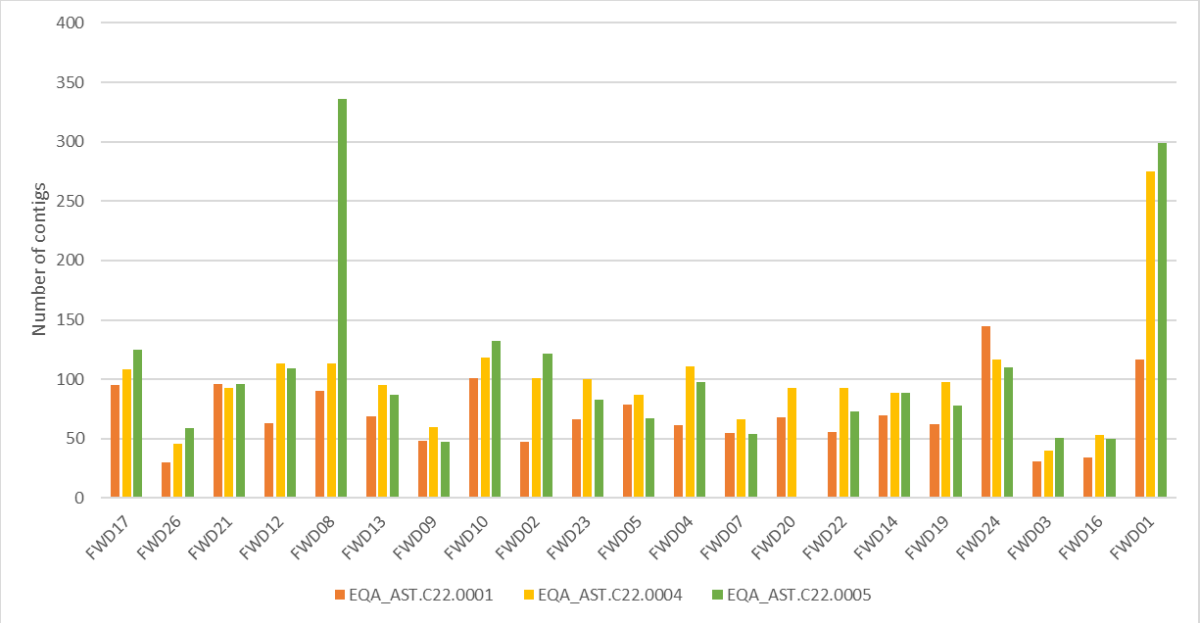

Figure 7. Total number of contigs in Campylobacter strains: orange – EQA_AST.C22.0001, yellow – EQA_AST.C22.0004 and green – EQA_AST.S22.0005. No data available for strain EQA_AST.C22.0005 for laboratory FWD20 due to failing of sequencing.

The N50 value, defined as the shortest contig length at 50% of the total assembly length, varied between almost all the laboratories from approximately 15,000 bp to 160,000 bp. An exception was laboratory FWD07 that reported remarkably high N50 number, reaching approximately 900,000 bp. Participant FWD07 was the only participant using CLC for assembly which might explain the high N50. Moreover, it is not known which sequencing method was used in the participants laboratory, for example whether a long-read chemistry was used. In general, the higher the N50 number, the longer the contigs and therefore, the better an assembly is.

Figure 8. The N50 value for all three strains: orange – EQA_AST.C22.0001, yellow – EQA_AST.C22.0004 and green – EQA_AST.C22.0005. No data available for strain EQA_AST.C22.0005 for laboratory FWD20 due to failing of sequencing.

Overall, the qualities of the sequences and assemblies submitted by the participants were satisfactory to identify resistance genes and point mutations. There is no golden standard as to minimum requirements for reliable antimicrobial resistance gene detection

and point mutation identification, however, some suggestions can be found in literature. Some authors suggest, among other parameters, N50 of >30000 bp and a maximum number of contigs of 500 (Bortolaia et al., 2020).

4.2. AMR gene and PMs detection methods used

Similarly as for *Salmonella*, the methods used by the participants varied a lot, however the majority of participants used the ResFinder database, either on assemblies or using raw reads. Different tools were used to query or map to the database. ResFinder as a tool was used by six participating laboratories alone and by 10 laboratories in combination with another tool. Figure 9 shows different tools used by the participants for antimicrobial resistance gene detection.

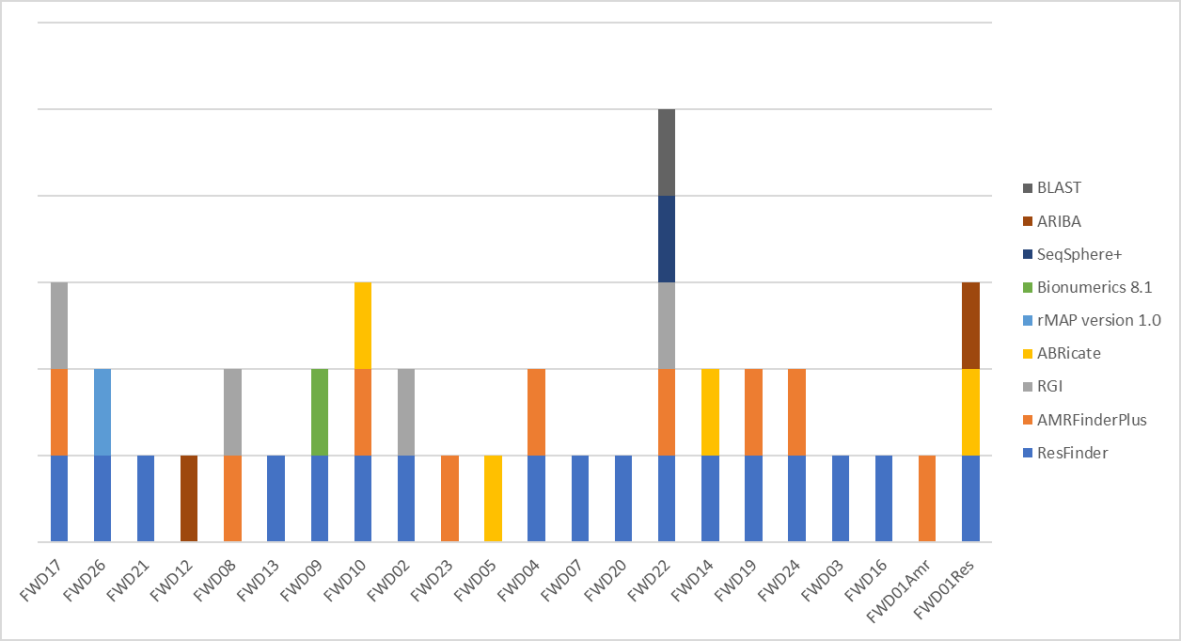

Figure 9. Tools used by the participants for AMR gene detection for all *Campylobacter* strains.

The second most used database was AMRFinderPlus, and the input for analysis in AMRFinderPlus was assemblies primarily made with SPAdes or SKESA. AMRFinder was used by one laboratory as the only tool and by seven laboratories in combination with other methods. The vast majority of participants only used assembly based methods for detection of AMR genes and the most popular assembler was SPAdes used by 8 participants followed by SKESA used by 5 participants. As mentioned in section 3.2 the SAUTE assembler could be considered for assemblies instead of SKESA. Other participants used Velvet, CLC Genomics, Unicycler or Shovill.

The tools used for point mutations detection for all participants are summarized in Figure 10.

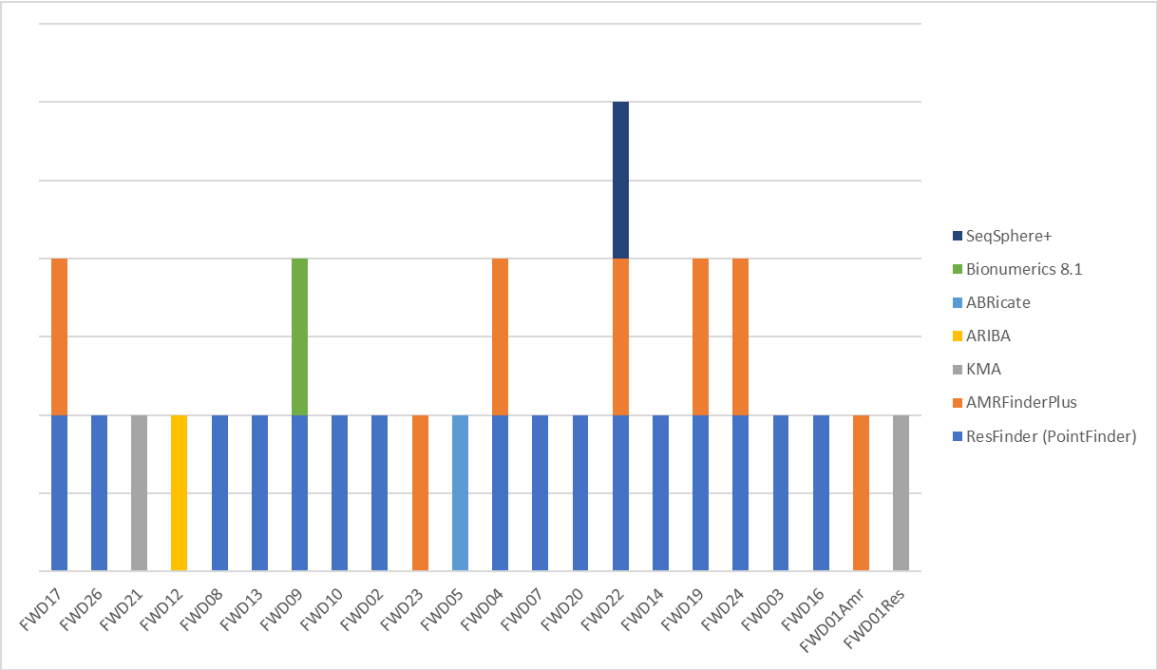

Figure 10. Overview of tools used for point mutation detection by all participants for all *Campylobacter* strains.

Here, PointFinder was the preferred tool, as 10 participants used it as a stand-alone tool and 6 participants in combination with other methods. AMRFinderPlus was the second most popular tool, being used by 6 participants in total, one of which used it as the only tool.

4.3. Species and STs reported

All the participants correctly reported the species of all *Campylobacter* strains. All but one participants reported the correct STs. Participant FWD05 reported the wrong ST for all three strains, even though they reported using PubMLST to identify the ST. For the correct species and ST of the test strains, see Table 3.

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

In this section, we used two reference data sets for comparison, as explained in Table 5. In the following strain-specific tables, the expected antibiotic resistance genes for each strain are marked with an "X" in the columns FWD01Res and FWD01Amr. The participant laboratories are grouped into four categories. The first two categories are based on the two main tools used to identify the antibiotic resistance genes: ResFinder (Green) and AMRFinder (Orange), together with the corresponding reference datasets (FWD01Res and FWD01Amr). Participants that have used more than one tool are grouped in the "Mixed methods" (Yellow) category. The "Other" (Blue) category indicates the participants that used a single other tool. This was ARIBA and ABRicate for laboratories FWD12 and FWD05, respectively. For overview of tools used by each laboratory for antibiotic gene detection, see Figure 9.

The expected point mutations are also marked with an "X" in separate strain-specific tables in columns FWD01Amr and FWD01Res. The participants are grouped into the same categories as for the antibiotic gene detection. For an overview of which tools different participants used for point mutation detection, see Figure 10.

4.4.1. Strain EQA_AST.C22.0001

Strain EQA_AST.C22.0001 is a *C. jejuni* strain, ST 7433.

Table 13. Genes found in strain EQA_AST.C22.0001, Green – ResFinder, Red – AMRFinder, Yellow – mixed methods, Blue – single other method different from ResFinder and AMRFinder. Columns with reference results, FWD01Res and FWD01Amr, are highlighted in light grey.

Lab#	FWD01Res	FWD17	FWD21	FWD13	FWD09	FWD07	FWD20	FWD03	FWD16	FWD01Amr	FWD23	FWD26	FWD08	FWD10	FWD02	FWD04	FWD22	FWD14	FWD19	FWD24	FWD12	FWD05
	ResFinder									AMRFinder		Mixed methods									Other	
aad9										X		X					X				X	
aph(2'')-If	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
aph(3') *				X																		
aph(3´)-III	X	X	X		X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
blaOXA-193		X		X	X	X		X	X	X		X	X	X	X	X	X	X		X	X	X
blaOXA-450															X							
blaOXA-451															X							
blaOXA-452															X							
blaOXA-453															X							
blaOXA-489		X													X							
blaOXA-61	X	X	X	X			X								X			X		X	X	
blaOXA-660																					X	
cat	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
tet(O)	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

*** (Correct) gene family reported instead of gene**

The two reference datasets used, FWD01Res and FWD01Amr generated slightly different results with regard to antimicrobial genes detected. Genes *aad9* and *blaOXA-193* were identified by AMRFinderPlus, but not by ResFinder. On the other hand, gene *blaOXA-61* was detected by ResFinder, but not by AMRFinderPlus.

All participating laboratories identified the genes *aph(2")-If* and *tet(O)* and most of the participants reported the *aph(3')-III* gene.

One point mutation, *gyrA* T86I, was detected in both reference datasets, FWD01Res and FWDAmr. Another point mutation, in ribosomal protein L22, reported as *50S_L22_A103V*, was detected by the AMRFinderPlus only.

The gyrA T86I mutation was reported by almost all laboratories, apart from FWD05. The A103V mutation in the ribosomal protein L22 was reported by only five participants (out of 20). As described previously (Dahl et al., 2021), this mutation is equally common in resistant and sensitive isolates in a set of 516 clinical *Campylobacter* isolates. However, the authors classified this mutation as present, but not verified in relation to phenotype. This would justify why only some laboratories reported this mutation.

Table 14. Point mutations reported in strain EQA_AST.C22.0001, Green – ResFinder, Red – AMRFinder, Yellow – mixed methods, Blue – single other method different from ResFinder and AMRFinder. Columns with reference results, FWD01Res and FWD01Amr, are highlighted in light grey.

Lab#	FWD26	FWD08	FWD13	FWD09	FWD10	FWD02	FWD07	FWD20	FWD14	FWD03	FWD16	FWD01Amr	FWD23	FWD17	FWD04	FWD22	FWD19	FWD24	FWD01Res	FWD21	FWD12	FWD05
	ResFinder											AMRFinder		Mixed methods					Other			
gyrA T86I	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
gyrA_2 C86I			X																			
50S_L22 A103V	X			X								X			X	X				X		

Laboratory FWD09 reported additionally as many as 26 point mutations in strain EQA_AST.C22.0001 as the only participant (Table S1). These nucleotide substitutions were in the *gyrA* gene (N=23) and in *rpsL* (N=3). Similarly, laboratory FWD10 reported, as the only participant, four unique mutations in *gyrA* gene and four in *cmeR* genes.

4.4.2. Strain EQA_AST.C22.0004

Strain EQA_AST.C22.0004 is a *Campylobacter coli* strain, ST872.

Table 15. Genes found in strain EQA_AST.C22.0004, Green – ResFinder, Red – AMRFinder, Yellow – mixed methods, Blue – single other method different from ResFinder and AMRFinder. Columns with reference results, FWD01Res and FWD01Amr, are highlighted in light grey.

Lab#	FWD01Res	FWD17	FWD21	FWD13	FWD09	FWD07	FWD20	FWD03	FWD16	FWD01Amr	FWD23	FWD26	FWD08	FWD10	FWD02	FWD04	FWD22	FWD14	FWD19	FWD24	FWD12	FWD05
	ResFinder									AMRFinder		Mixed methods									Other	
aac(6')-aph(2'')	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
aadE										X	X	X		X		X	X		X	X	X	
ant(6)-Ia *	X	X	X	X	X	X	X	X	X	X		X			X	X		X		X	X	X
aph(3') **				X									X									
aph(3´)-III	X	X	X		X	X		X	X	X	X	X		X	X	X	X	X	X	X	X	X
blaOXA-193		X			X	X		X	X	X			X	X	X	X	X	X		X	X	X
blaOXA-45															X							
blaOXA-451															X							
blaOXA-452															X							
blaOXA-453															X							
blaOXA-489		X													X							
blaOXA-61	X	X	X	X			X								X			X		X	X	
sat4										X		X	X	X		X	X		X	X	X	
tet(O)	X		X	X	X		X	X	X	X	X		X	X	X		X	X		X	X	

* - synonym of aadE
** - (Correct) gene family reported instead of gene

Differences were observed between the two reference datasets, FWD01Res and FWD01Amr. The following genes were identified by AMRFinderPlus and not by ResFinder: *blaOXA-193* and *sat4*.

All participants identified the *aac(6')-aph(2'')* gene, coding for a bifunctional aminoglycoside-modifying enzyme (Qin et al., 2012). Almost all participants, apart from one laboratory, reported the gene *ant(6)-Ia* (or its synonym *aadE*), coding for aminoglycoside adenyltransferase, responsible for streptomycin resistance (Hormeño et al., 2018).

Table 16. Point mutations reported in strain EQA_AST.C22.0004, Green – ResFinder, Red – AMRFinder, Yellow – mixed methods, Blue – single other method different from ResFinder and AMRFinder. Columns with reference results, FWD01Res and FWD01Amr, are highlighted in light grey.

Lab#	FWD26	FWD08	FWD13	FWD09	FWD10	FWD02	FWD07	FWD20	FWD14	FWD16	FWD03	FWD01Amr	FWD23	FWD17	FWD04	FWD22	FWD19	FWD24	FWD01Res	FWD21	FWD12	FWD05
	ResFinder											AMRFinder		Mixed methods					Other			
gyrA T86I	X	X				X	X	X				X	X		X	X	X		X	X	X	
gyrA_2(p.T86I)			X	X	X				X	X				X								

The *gyrA* T86I substitution was reported by 17 out of 20 participants in strain EQA_AST.C22.0004. It is worth mentioning that 6 out of those 16 participants did report this mutation as present in the *gyrA*_2 variant of the gene, present in PointFinder database since June 2022. All these latter participants used PointFinder as the detection tool (FWD17 in combination with another tool).

Laboratory FWD09, similarly as for the previous strain, reported many nucleotide substitutions, mostly in 23S ribosomal RNA gene (13), but also *rpsL* and *gyrA*. Laboratory FWD21 also reported four additional unique mutations in *gyrA* and two in *cmeR* gene (Table S2).

4.4.3. Strain EQA_AST.C22.0005

Strain EQA_AST.C22.0005 is a *Campylobacter coli* strain, ST872.

Table 17. Genes found in strain EQA_AST.C22.0005, Green – ResFinder, Red – AMRFinder, Yellow – mixed methods, Blue – single other method different from ResFinder and AMRFinder. Columns with reference results, FWD01Res and FWD01Amr, are highlighted in light grey.

Lab#	FWD01Res	FWD17	FWD21	FWD13	FWD09	FWD07	FWD03	FWD16	FWD01Amr	FWD23	FWD26	FWD08	FWD10	FWD02	FWD04	FWD22	FWD14	FWD19	FWD24	FWD12	FWD05
	ResFinder								AMRFinder		Mixed methods									Other	
aadE*																				X	
aadE-Cc	X	X	X		X	X	X	X	X		X		X	X	X	X	X	X	X		X
ant(6)-Ig																				X	
blaOXA-489	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X
blaOXA-61																				X	
blaOXA-66																				X	
tet(O)	X	X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X

*Correct gene identified, but likely with a typo

In strain EQA_AST.C22.0005 the same genes were identified in the two reference sets FWD01Res and FWD01Amr: *aadE-Cc*, *blaOXA-489* and *tet(O)*. The same genes were identified by most of the participants. Laboratory FWD12 identified additionally genes *ant(6)-Ig*, *blaOXA-61* and *blaOXA-66*.

Majority of participants reported the A2075G nucleotide substitution in the 23S rRNA gene. The T86I mutation in *gyrA* gene (or *gyrA*_2 variant) was also reported by the majority of participants. Additionally, laboratory FWD21 reported mutations in *cme* genes and other mutations in *gyrA* gene.

Table 18. Point mutations reported in strain EQA_AST.C22.0005, Green – ResFinder, Red – AMRFinder, Yellow – mixed methods, Blue – single other method different from ResFinder and AMRFinder. Columns with reference results, FWD01Res and FWD01Amr, are highlighted in light grey.

Lab#	FWD26	FWD08	FWD13	FWD09	FWD10	FWD02	FWD07	FWD14	FWD03	FWD16	FWD01Amr	FWD23	FWD17	FWD04	FWD22	FWD19	FWD24	FWD01Res	FWD21	FWD12	FWD05
	ResFinder										AMRFinder		Mixed						Other		
gyrA T86I	X	X					X				X	X		X	X	X		X	X	X	
gyrA_2 T86I			X	X	X			X		X			X								
23S_A2075G	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	

*No data available due to failing in sequencing

Additionally, laboratory FWD09 reported 20 point mutations in genes *rpsL*, 23S and *gyrA* (Table S3).

5. CONCLUSIONS

This EQA1-WGS-AMR, organised by Statens Serum Institut (SSI), is the first exercise in the FWDAMR-RefLabCap project, in a series of three, spanning over 3 years. The aim of this and following EQAs is to support the further development and implementation of the standard protocol for AMR gene detection and clone identification (https://www.fwdamr-reflabcap.eu/resources/protocols-and-guidelines).

Thirty-one participants accepted the invitation and 25 participants submitted the results. The reasons for 6 countries withdrawing from submitting the results throughout the duration of the EQA were mainly problems with delivery of specific reagents for sequencing, delay in materials supply, issues with DNA extraction and purification problems or lack of experience in analysing this type of data.

EQA1-WGS-AMR participants extracted DNA from the received isolates, performed Whole Genome Sequencing and identified antimicrobial resistance genes and point mutations. Overall, the participants performed well and identified the expected targets. This EQA exercise highlighted that small differences in the results are to be expected among participants likely due to using different input data types (raw reads or assemblies), tools used to perform assemblies and, finally, tools used to detect genes and point mutations. Different tools can give different results. Whether using mapping of raw reads or blasting of assemblies, there are different points of attention to consider. If there is a gene in a genomic region that is difficult to assemble, it could potentially be missed in an assembly based approach. If there are many closely related variants in the database used, it might complicate determination of the exact variant through mapping.

Reference gene databases can also give different results. Some genes have different nomeclature in different databases, some databases are based on amino acids and some on nucleotides. Finally, it is always important to ensure that the latest version of the database is being used, as well as to ensure that the database is regularly updated. Find more information on databases and tools in the protocol (https://www.fwdamr-reflabcap.eu/resources/protocols-and-guidelines).

In this EQA, an example of how presence or absence of certain genes in some databases but not the others can affect the results was the *qacL* gene, present in AMRFinderPlus database, but not in ResFinder database.

For the next round of EQA, EQA2-WGS-AMR in 2023, the provider is planning to redesign the questionnaire, modify questions used for gene reporting and add additional questions about methods used.

6. REFERENCES

Bharat, A., Petkau, A., Avery, B. P., Chen, J., Folster, J., Carson, C. A., Kearney, A., Nadon, C., Mabon, P., Thiessen, J., Alexander, D. C., Allen, V., El Bailey, S., Bekal, S., German, G. J., Haldane, D., Hoang, L., Chui, L., Minion, J., … Mulvey, M. R. (2022). Correlation between Phenotypic and In Silico Detection of Antimicrobial Resistance in Salmonella enterica in Canada Using Staramr. *Microorganisms*, *10*(2), 1–10. https://doi.org/10.3390/microorganisms10020292

Bortolaia, V., Kaas, R. S., Ruppe, E., Roberts, M. C., Schwarz, S., Cattoir, V., Philippon, A., Allesoe, R. L., Rebelo, A. R., Florensa, A. F., Fagelhauer, L., Chakraborty, T., Neumann, B., Werner, G., Bender, J. K., Stingl, K., Nguyen, M., Coppens, J., Xavier, B. B., … Aarestrup, F. M. (2020). ResFinder 4.0 for predictions of phenotypes from genotypes. *Journal of Antimicrobial Chemotherapy*, *75*(12), 3491–3500. https://doi.org/10.1093/jac/dkaa345

Ceccarelli, D., Salvia, A. M., Sami, J., Cappuccinelli, P., & Colombo, M. M. (2006). New cluster of plasmid-located class 1 integrons in Vibrio cholerae O1 and a dfrA15 cassette-containing integron in Vibrio parahaemolyticus isolated in Angola. *Antimicrobial Agents and Chemotherapy*, *50*(7), 2493–2499. https://doi.org/10.1128/AAC.01310-05

Chang, M. X., Zhang, J. F., Sun, Y. H., Li, R. S., Lin, X. L., Yang, L., Webber, M. A., & Jiang, H. X. (2021). Contribution of Different Mechanisms to Ciprofloxacin Resistance in Salmonella spp. *Frontiers in Microbiology*, *12*(May). https://doi.org/10.3389/fmicb.2021.663731

Dahl, L. G., Joensen, K. G., Østerlund, M. T., Kiil, K., & Nielsen, E. M. (2021). Prediction of antimicrobial resistance in clinical Campylobacter jejuni isolates from whole-genome sequencing data. *European Journal of Clinical Microbiology and Infectious Diseases*, *40*(4), 673–682. https://doi.org/10.1007/s10096-020-04043-y

Eaves, D. J., Randall, L., Gray, D. T., Buckley, A., Woodward, M. J., White, A. P., & Piddock, L. J. V. (2004). Prevalence of mutations within the quinolone resistance-determining region of gyrA, gyrB, parC, and parE and association with antibiotic resistance in quinolone-resistant Salmonella enterica. *Antimicrobial Agents and Chemotherapy*, *48*(10), 4012–4015. https://doi.org/10.1128/AAC.48.10.4012-4015.2004

Hormeño, L., Ugarte-Ruiz, M., Palomo, G., Borge, C., Florez-Cuadrado, D., Vadillo, S., Píriz, S., Domínguez, L., Campos, M. J., & Quesada, A. (2018). Ant(6)-I genes encoding aminoglycoside O-nucleotidyltransferases are widely spread Among streptomycin resistant strains of Campylobacter jejuni and Campylobacter coli. *Frontiers in Microbiology*, *9*(OCT), 1–8. https://doi.org/10.3389/fmicb.2018.02515

Magnet, S., Courvalin, P., & Lambert, T. (1999). Activation of the cryptic aac(6')-Iy aminoglycoside resistance gene of Salmonella by a chromosomal deletion generating a transcriptional fusion. *Journal of Bacteriology*, *181*(21), 6650–6655. https://doi.org/10.1128/jb.181.21.6650-6655.1999

Qin, S., Wang, Y., Zhang, Q., Chen, X., Shen, Z., Deng, F., Wu, C., & Shen, J. (2012). Identification of a novel genomic island conferring resistance to multiple aminoglycoside antibiotics in Campylobacter coli. *Antimicrobial Agents and Chemotherapy*, *56*(10), 5332–5339. https://doi.org/10.1128/AAC.00809-12

Souvorov, A., & Agarwala, R. (2021). SAUTE: sequence assembly using target enrichment. *BMC Bioinformatics*, *22*(1), 1–22. https://doi.org/10.1186/s12859-021-04174-9

7. ANNEX

Table S1. Unique point mutations reported by two participants in *Campylobacter* strain EQA_AST.C22.0001

Laboratory	Gene	Point mutations reported
FWD09	*gyrA*	dA64G, dT72C, dC243T, dC257T, dT357C, dC360T, dC471T, dT483C, dA608G, dC622T, dA738C, dC786A, dG854A, dT957C, dC975T, dT1017C, dG1047A, dT1071C, dA1233G, dC1266G, dC1476T, dC1530T, dA1533G
	rpsL	dC201T, dG222A, dT363C
FWD10	*gyrA*	Q863*, R285K, N203S, S22G
	cmeR	G144D, S207G, P183R, T6I

Table S2. Unique point mutations reported by two participants in *Campylobacter* strain EQA_AST.C22.0004

Laboratory	Gene	Point mutations reported
FWD09	*rpsL*	dT363C
	23S	dC296G, dG364C, dA554C, dT571G, dG402A, dT416G, dC418T, dA1722C, dT1727C, dT1744C, A1751G, dG1753A, dC2105T
FWD21	*gyrA*	gyrA_70, gyrA_85, gyrA_90, gyrA_104
	cmeR	cmeR_AND_cmeABC_86, cmeR_AND_cmeABC_696

Table S3. Unique point mutations reported by two participants in *Campylobacter* strain EQA_AST.C22.0005

Laboratory	Gene	Point mutations reported
FWD09	*gyrA*	dC257T
	rpsL	dC201T, dT363C, dC102T, dT351C, dT354C, dG376A, pA126T
	23S	dC296G, dG364C, dA554C, dT571G, dC418T, dA1722C, dT1727C, dT1744C, dA1751G, dG1753A, dC2105T, dA2067G
FWD10	*rpsL*	pA126T
FWD21	*gyrA*	gyrA_70, gyrA_85, gyrA_86, gyrA_90, gyrA_104
	cmeR	cmeR_AND_cmeABC_696

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