FWD AMR· RefLabCap

EQA1-WGS-AMR and RingTrial1-WGS-AMR results

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Outline

- Introduction about FWD AMR-RefLabCap
- EQA1-WGS-AMR
  - Sequencing QC
  - Tools and databases
  - Result examples
- RingTrial1
  - Tools and databases
  - Result examples
- Conclusions, lessons learned and future plans

FWD AMR-RefLabCap

Provision of EU networking and support for public health reference laboratory functions for antimicrobial resistance in *Salmonella* and *Campylobacter* in human samples

- The project is run under a contract with HaDEA on behalf of DG SANTE and in close cooperation with ECDC
- 4-year project: 2021-2024

- Contractors:
  - Statens Serum Institut (SSI)
    - Project leader: Eva Møller Nielsen, Section of Foodborne Infections
  - National Food Institute, Technical University of Denmark (DTU)
    - René Hendriksen and Birgitte Helwigh, Research group for global capacity building

Support countries to enhance the validity and accuracy of surveillance data in order to inform concerted actions against AMR at EU level and enable better detection and control of cross border threats to human health from AMR

Project team

- Statens Serum Institut (SSI)
  - Eva Møller Nielsen: Project manager
  - Egle Kudirkiene: Priority countries
  - Susanne Schjørring: Network tasks
  - Eva Litrup: Methods tasks
  - Other team members
    - Jeppe Boel
    - Malgorzata Ligowska-Marzeta
    - Mia Torpdahl
    - Karen Loaiza Conza
- Technical University of Denmark (DTU)
  - René Hendriksen: Training tasks
  - Birgitte Helwigh: Coordination of DTU activities
  - Other team members
    - Ana Rita Bastos Rebelo
    - Jette Sejer Kjeldgaard
    - Susanne Karlsmose Pedersen

The tasks are organised in groups/teams:

- Management team
- NRL Network team
- Training team
- Methods team
- Priority countries team

EQA vs RingTrial in FWD AMR-RefLabCap

EQA1-WGS-AMR

To evaluate and ensure the quality and comparability of the WGS-based data produced by the NRLs

Live bacteria
DNA purification + WGS required

RingTrial1

To investigate the outcome of different databases, tools and bioinformatic pipelines used by NRLs and enable comparison of their performance in AMR gene and point mutation detection

Analyze provided sequences

EQA1-WGS-AMR - First annual *in vitro* external quality assessement scheme for WGS-based resistome profiling of *Salmonella* and *Campylobacter*

- 39 participants invited
- 25 participants submitted results
- 3 *Salmonella* and 3 *Campylobacter* strains
- Participants were asked to sequence strains and analyse sequences for AMR genes and point mutations
- Reporting results through an online platform
- Aim of this and following EQAs is to support the further development and implementation of WGS in the NRLs and evaluate the quality and comparability of the WGS-based data produced

Salmonella and *Campylobacter* strain characteristics

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. 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

Sequencing QC - *Salmonella*

Genome size

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.

Contigs

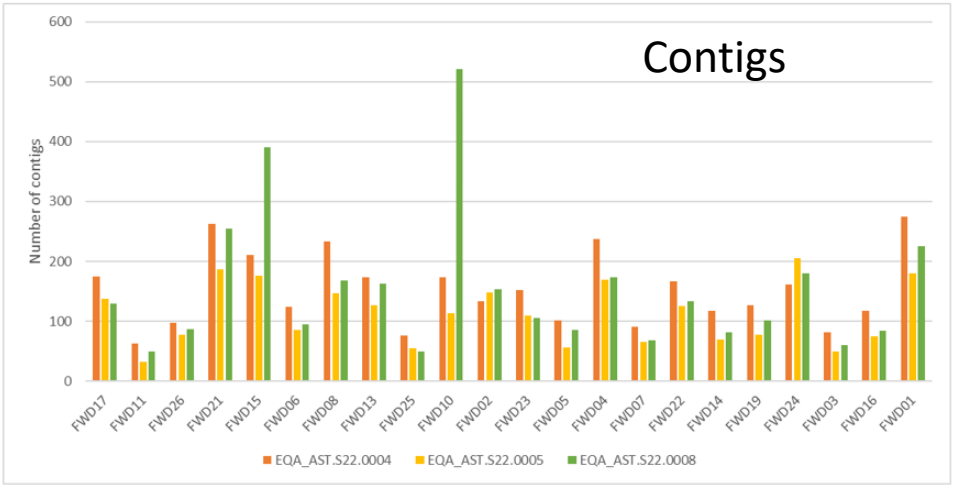


Figure 2. Total number of contigs in Salmonella strains: orange – EQA_AST.S22.0004, yellow – EQA_AST.S22.0005 and green – EQA_AST.S22.0008.

N50

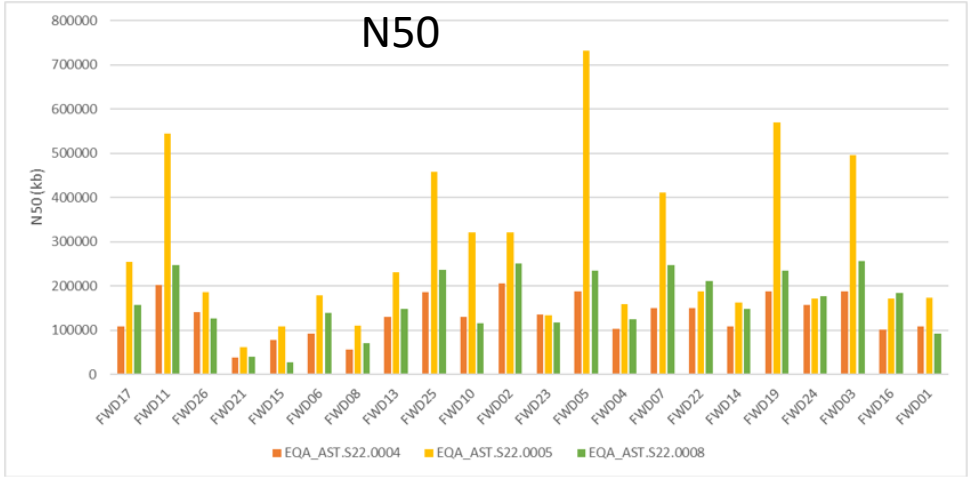


Figure 3. The N50 value for all three strains: orange – EQA_AST.S22.0004, yellow – EQA_AST.S22.0005 and green – EQA_AST.S22.0008.

Sequencing QC - *Campylobacter*

Genome size

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.

Contigs

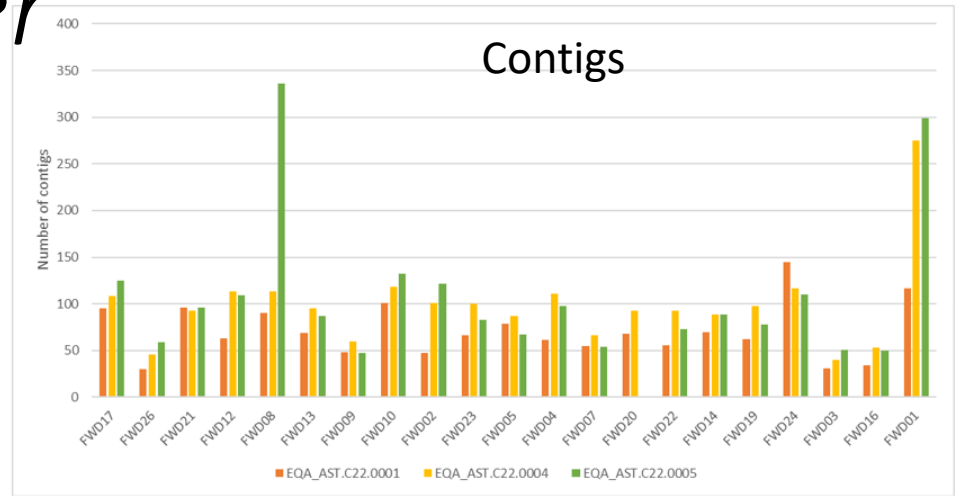


Figure 7. Total number of contigs in Campylobacter strains: orange – EQA_AST.C22.0001, yellow – EQA_AST.C22.0004 and green – EQA_AST.S22.0005. No data available for strain EQA_AST.C22.0005 for laboratory FWD20 due to failing of sequencing.

N50

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.

Tools and Databases

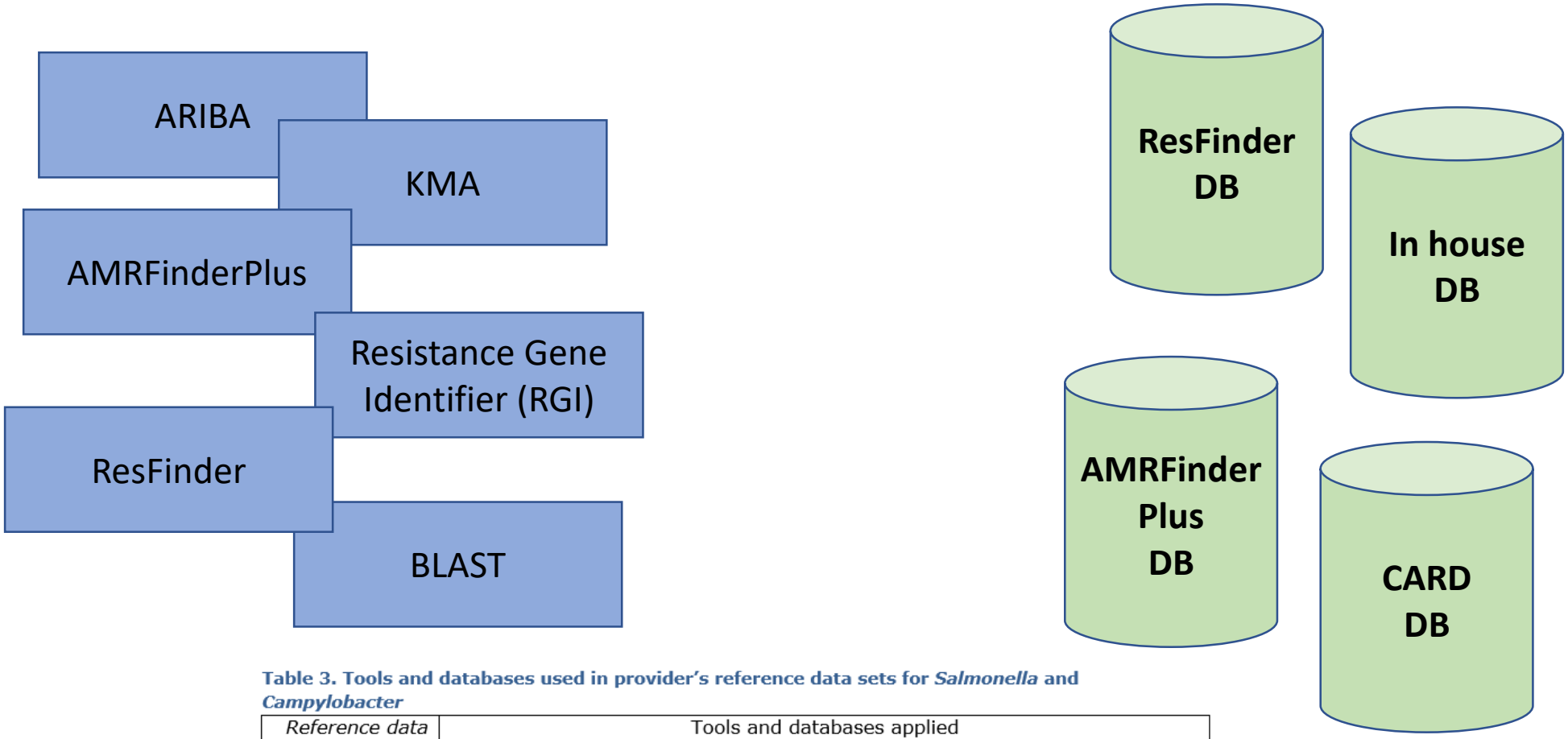


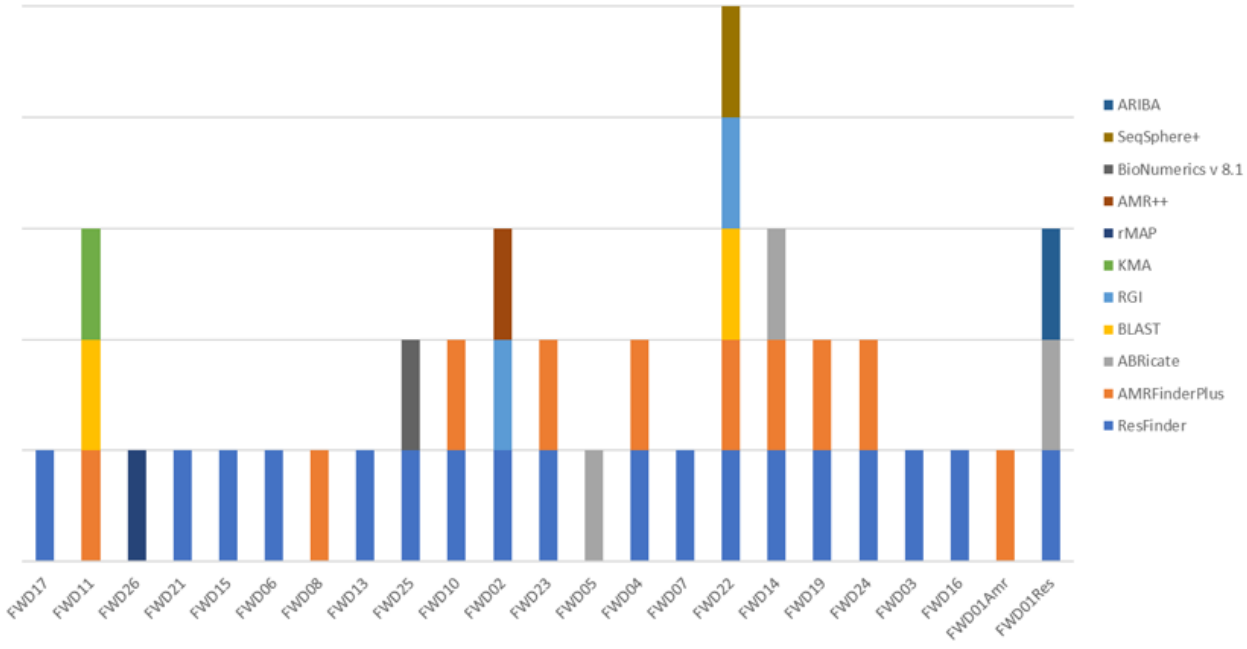
Table 3. Tools and databases used in provider's reference data sets for *Salmonella* and *Campylobacter*

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*

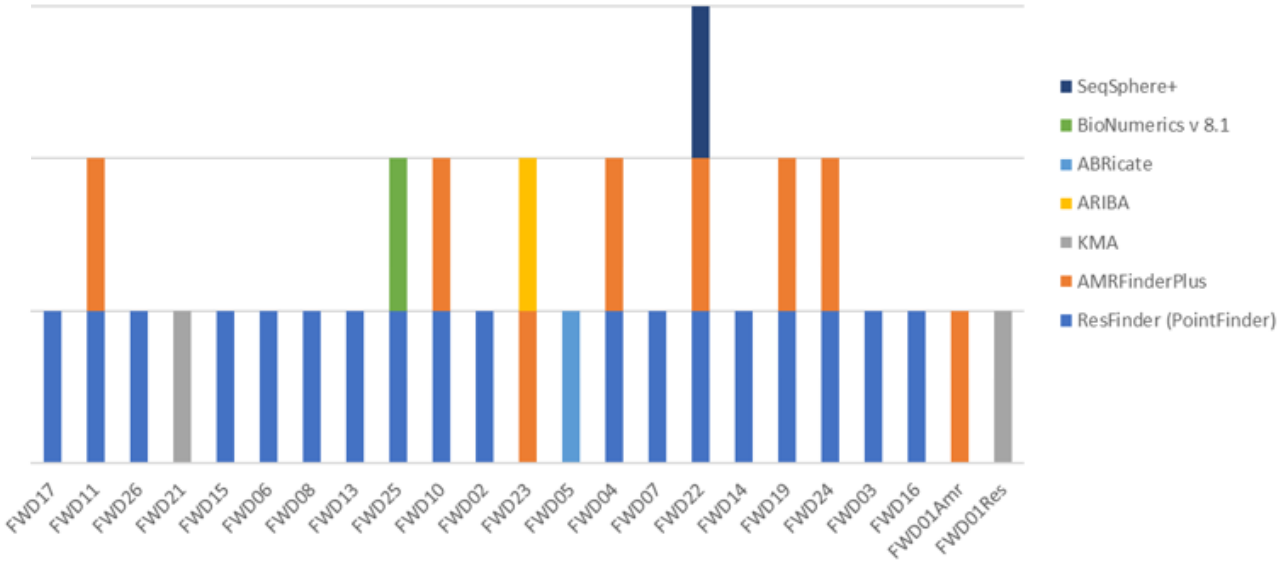
*Based on PointFinder database

Tools used for *Salmonella* gene and point mutation detection

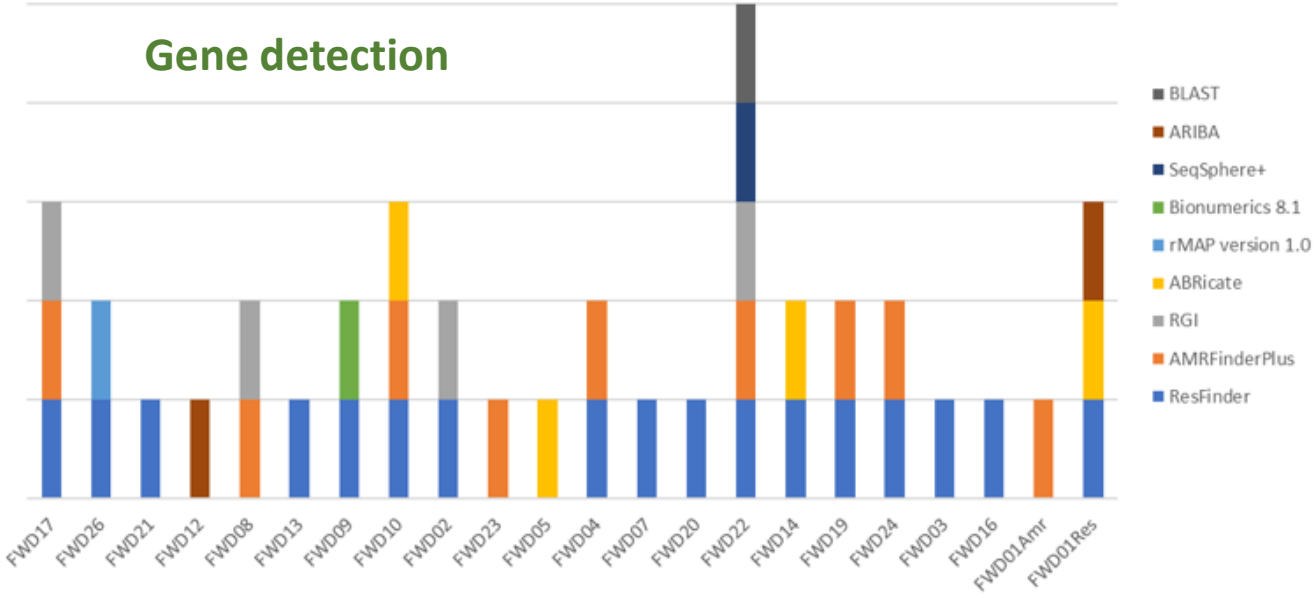
Gene detection

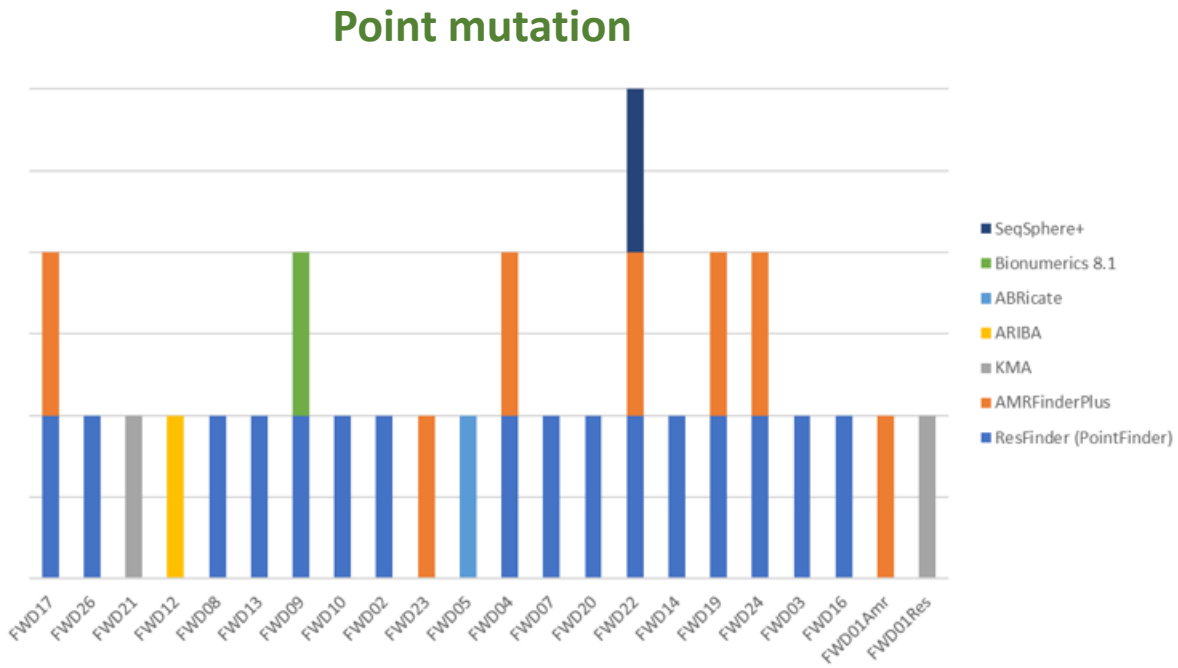


Point mutation identification



Tools used for *Campylobacter* gene and point mutation detection





Results – genes *Salmonella*

Table 5. Genes found in strain EQA_AST.S22.0004, Green – ResFinder, Red – AMRFinder, Yellow – mixed methods, Blue – single other method different from ResFinder and AMRFinder.

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

Results – genes *Salmonella*

Table 7. Genes found in strain EQA_AST.S22.0005, Green – ResFinder, Red – AMRFinder, Yellow – mixed methods, Blue – single other method different from ResFinder and AMRFinder.

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

Results – PM *Salmonella*

Table 8. 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.

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.

Results – genes *Campylobacter*

Table 15. Genes found in strain EQA_AST.C22.0005, Green – ResFinder, Red – AMRFinder, Yellow – mixed methods, Blue – single other method different from ResFinder and AMRFinder.

Lab#	FW D01Res	FW D17	FW D21	FW D13	FW D09	FW D07	FW D03	FW D16	FW D01Amr	FW D23	FW D26	FW D08	FW D10	FW D02	FW D04	FW D22	FW D14	FW D19	FW D24	FW D12	FW D05	FW D20
	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

Results – PM *Campylobacter*

Table 14. 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.

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).

RingTrial1 – First annual inter-laboratory ring-trial of bioinformatics pipelines for *Salmonella* and *Campylobacter*

- 39 participants invited, **23 participants submitted results**
- Whole Genome Sequences from 4 *Salmonella* and 4 *Campylobacter* strains (fasta or fastq format)
- Participants were asked to analyse sequences and report antimicrobial resistance genes and point mutations
- Reporting results through an online platform
- Aim of this and following ring-trials is to investigate outcome of different databases, tools and bioinformatic pipelines used by participants and compare their performance of detection of antimicrobial resistance genes and point mutations

Salmonella and *Campylobacter* strain characteristics

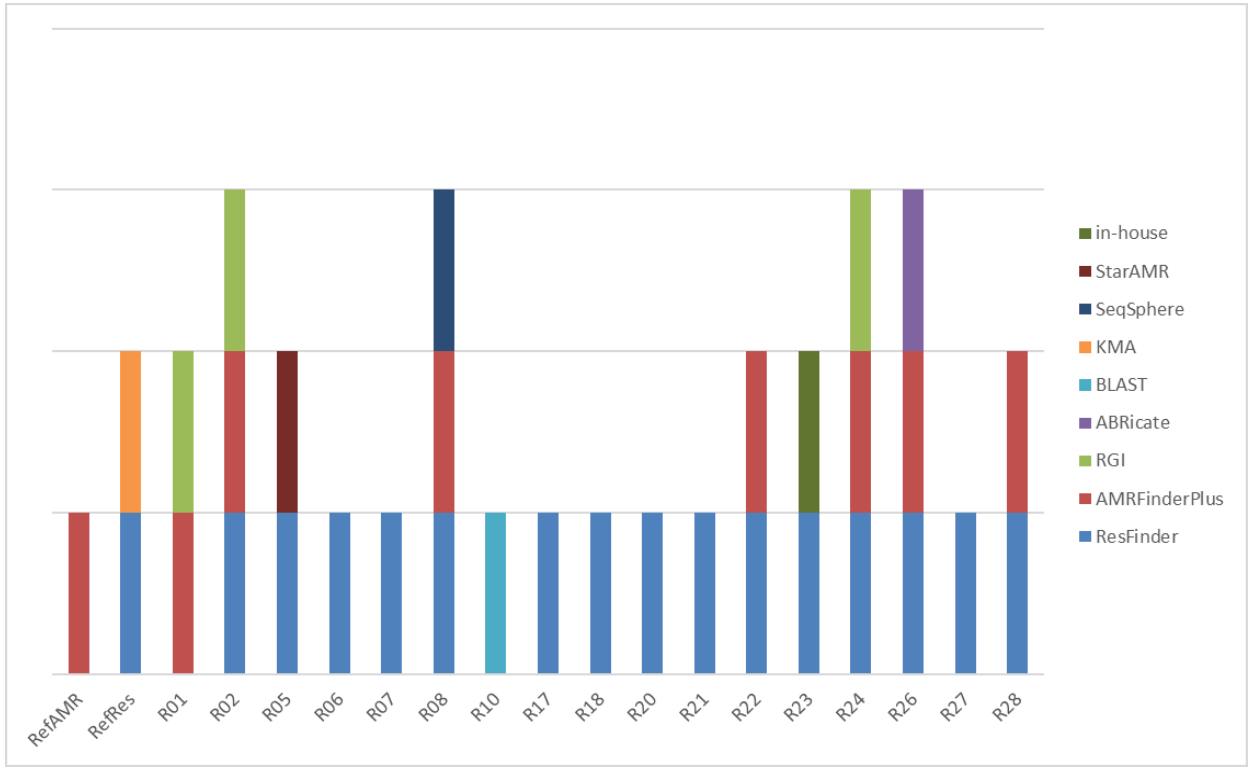
Sequence	Serotype	ST	Genes*	Point mutations
TRING1S-1	Bredeney	505	*qnrB19*	gyrA D87G
TRING1S-2	Monophasic Typhimurium (O5-)	34	*aac(3)-IVa, aadA16, aph(3'')-Ib, aph(4)-Ia, aph(6)-Id, arr-3, blaTEM-1, catA2, dfrA27, floR, qacEdelta1, sul1, sul2, tet(D)*	None
TRING1S-3	Corvallis	1541	*aph(3'')-Ib, aph(6)-Id, qnrS1, sul2, tet(A)*	None
TRING1S-4	Emek	76	*sul1*	gyrA S83Y

*AMRFinderPlus output

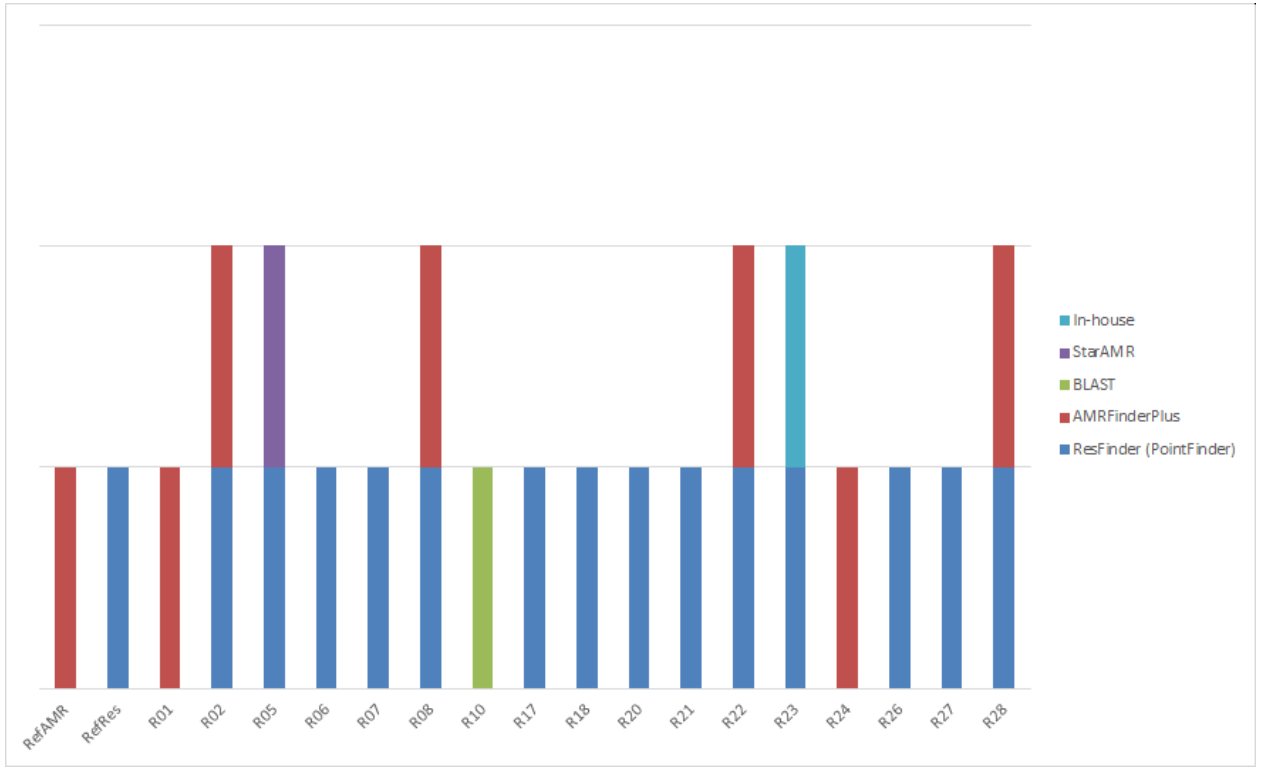
Sequence	Species	ST	Genes*	Point mutations
TRING1C-1	*C. jejuni*	19	*blaOXA-193*	gyrA T86I
TRING1C-2	*C. jejuni*	464	*blaOXA, tet(O)*	50S L22 A103V, gyrA T86I
TRING1C-3	*C. coli*	8195	*blaOXA-193, tet(O)*	gyrA T86I
TRING1C-4	*C. coli*	832	*aad9, aadE, blaOXA-193, tet(O)*	50S L22 A103V, gyrA T86I

*AMRFinderPlus output

Tools used for *Salmonella* gene and point mutation detection



Gene detection



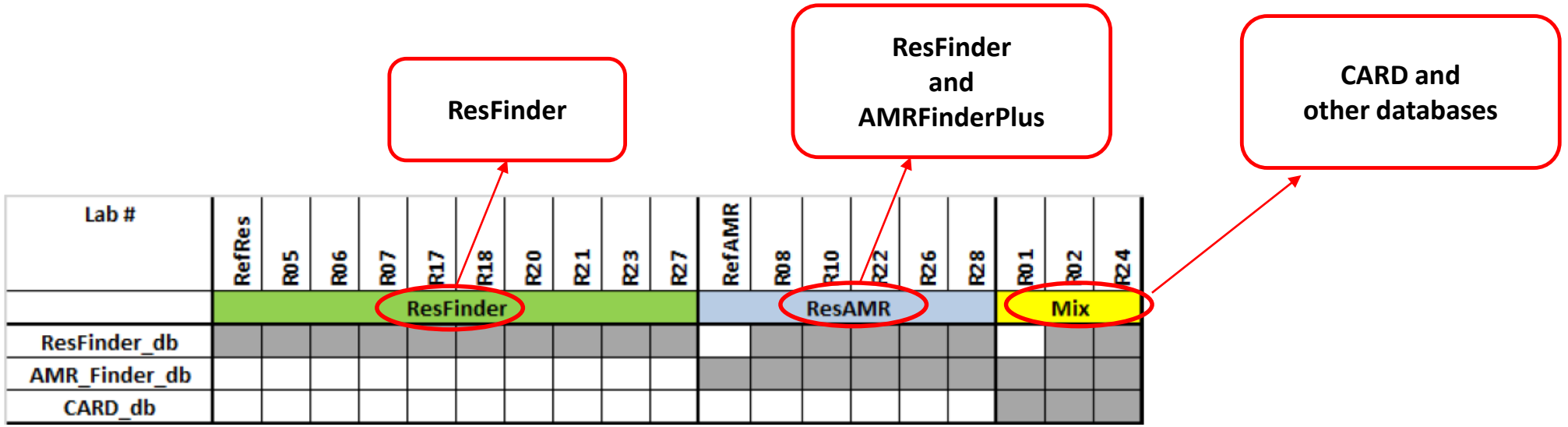
Point mutation identification

Reference data set name	Tools applied	
	Gene detection	**Point mutation identification**
RefAMR	AMRFinderPlus on SPAdes assemblies	AMRFinderPlus on SPAdes assemblies
RefRes	KMA with ResFinder database	KMA with PointFinder database

Salmonella gene and point mutation reporting

In general very good performance

Results classified according to databases or database combinations used:



One participant's result (R18) excluded in the analysis, likely due to submitting the results in the wrong order – shaded with grey in all tables

Salmonella TRING1S-1 - example

Cryptic gene, not present in all databases

Table 6. Genes found in sequence TRING1S-1, Green – ResFinder, Blue – AMRFinder and ResFinder, Yellow – a mix of databases.

Lab #	RefRes	R05	R06	R07	R17	R18	R20	R21	R23	R27	RefAMR	R08	R10	R22	R26	R28	R01	R02	R24
	ResFinder										ResAMR						Mix		
ResFinder_db																			
AMR_Finder_db																			
CARD_db																			
aac(6')-Iaa	X	X	X	X	X	X	X	X	X	X		X	X	X	X	X		X	X
qnrB19	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X
qnrB5																			X
qnrB81																			X
sul1						X													

Not present in AMRFinderPlus database (considered non-informative)

Table 7. Point mutation found in sequence TRING1S-1, Green – PointFinder, Blue – AMRFinder and Yellow – PointFinder and AMRFinderPlus databases.

Lab #	RefRes	R05	R06	R07	R10	R17	R18	R20	R21	R23	R26	R27	RefAMR	R01	R24	R02	R08	R22	R28
	ResFinder												AMRF			Mix			
ResFinder (PointFinder)																			
AMRFinderPlus																			
gyrA D87G	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X
gyrA S83Y							X												
parC T57S	X	X	X		X	X		X	X	X	X	X				X	X	X	

Salmonella TRING1S-2 - example

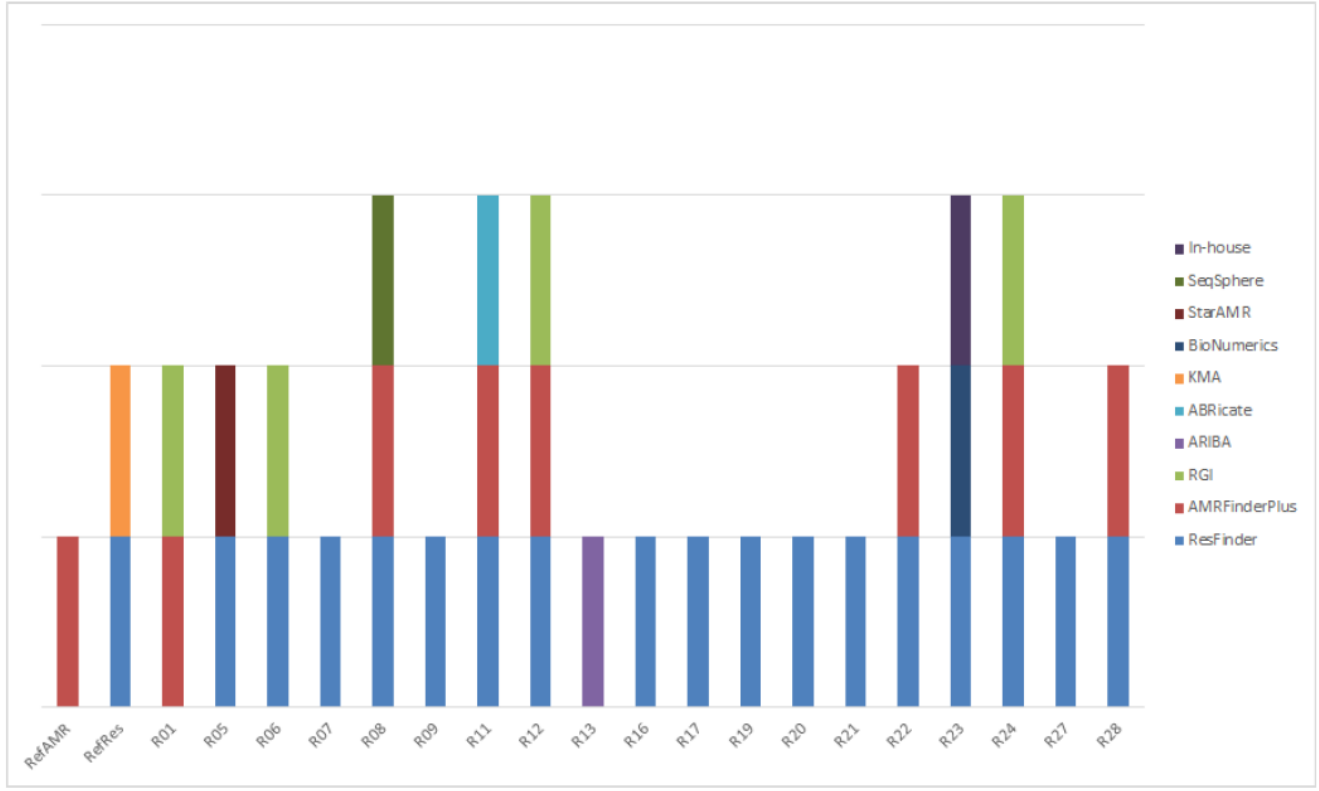
Table 8. Genes found in sequence TRING1S-2, Green – ResFinder, Blue – AMRFinder and ResFinder, Yellow – a mix of databases.

Lab #	RefRes	R05	R06	R07	R17	R18	R20	R21	R23	R27	RefAMR	R08	R10	R22	R26	R28	R01	R02	R24
	ResFinder										ResAMR						Mix		
ResFinder_db																			
AMRFinderPlus_db																			
CARD_db																			
aac(3)-IV	X	X	X	X	X		X	X	X	X		X	X		X			X	X
aac(3)-IVa											X	X		X		X			X
aac(3)-VIa																	X		
aac(6')-Iaa	X		X	X	X	X	X	X	X	X		X	X	X	X			X	X
aph(3'')-Ib		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
blaTEM-1B	X	X	X	X	X		X	X	X	X		X			X			X	X
qacE	X		X						X										
qacEdelta1											X				X	X		X	X
qnrS1						X													
sitABCD						X													
sul2	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X		X	
tet(A)						X													

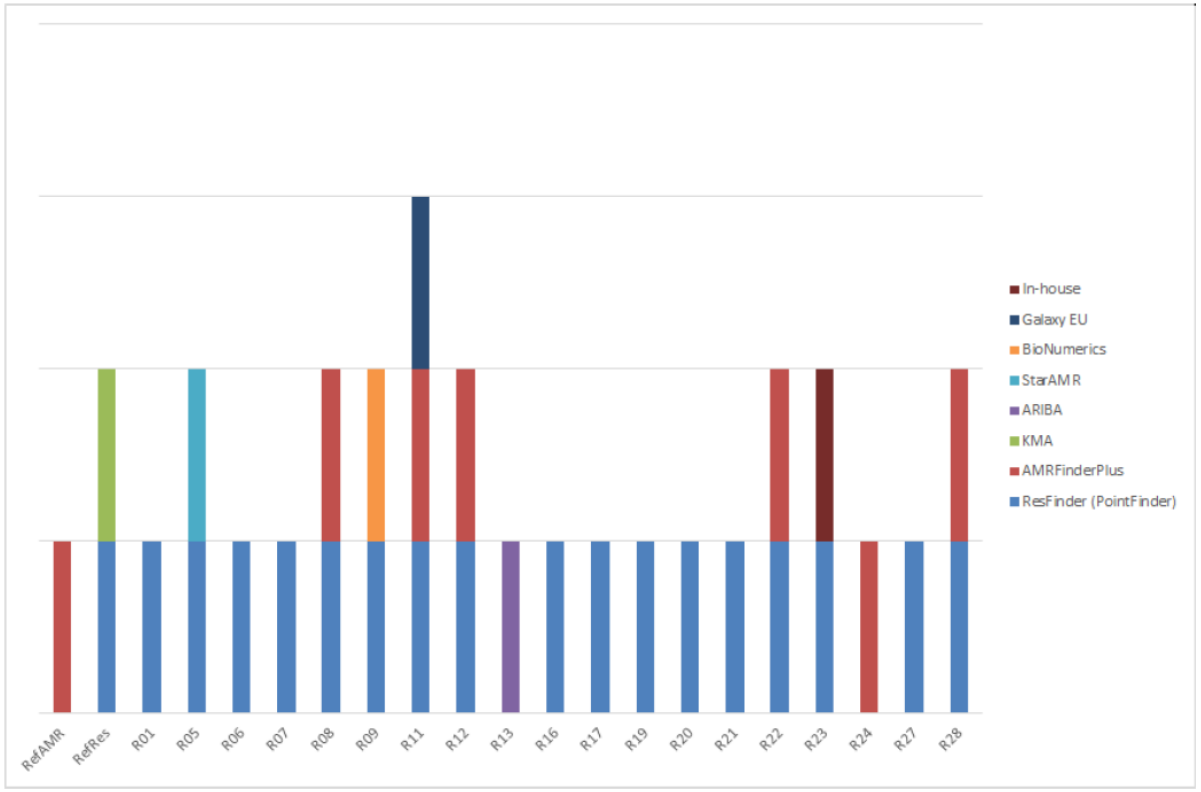
variant

mcr-9 does not confer resistance to colistin in over 100 natural *mcr-9*+ isolates
(Feldgarden et al 2022)

Tools used for *Campylobacter* gene and point mutation detection



Gene detection



Point mutation identification

Campylobacter TRING1C-1 – blaOXA example

Table 13. Genes found in sequence TRING1C-1, Green – ResFinder, Blue – AMRFinder + ResFinder, Yellow – CARD and other databases.

Lab #	RefRes	R05	R07	R09	R16	R17	R19	R20	R21	R23	R27	RefAMR	R08	R11	R22	R28	R06	R13*	R01	R12	R24
	ResFinder											ResAMR					Mix				
ResFinder																					
AMRFinderPlus																					
CARD																					
blaOXA					X																
blaOXA-193	X	X		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																	X
blaOXA-61		X	X	X														X		X	X
cmeABC+R																		X			
tet(O)																			X		

* used an in-house database

assemblies

reads

Campylobacter TRING1C-1 – blaOXA example

ResFinder output with reads

unknown beta-lactam	beta-lactam	Resistant	blaOXA-193 (blaOXA-193_CP013032)

ResFinder output with assembly

Antimicrobial	Class	WGS-predicted phenotype	Genetic background
ampicillin	beta-lactam	Resistant	blaOXA-61 (blaOXA-61_AY587956)
unknown beta-lactam	beta-lactam	Resistant	blaOXA-453 (blaOXA-453_KR061507), blaOXA-450 (blaOXA-450_KR061502), blaOXA-452 (blaOXA-452_KR061505), blaOXA-451 (blaOXA-451_KR061504), blaOXA-489 (blaOXA-489_CP013733), blaOXA-193 (blaOXA-193_CP013032)

Beta-lactam									
Resistance gene	Identity	Alignment Length/Gene Length	Position in reference	Contig or Depth	Position in contig	Phenotype	PMID	Accession no.	Notes
blaOXA-193	99.8708010336	774/774	1..774	NODE_3_length_154164_cov_96.482961	33734..34507	unknown beta-lactam	unpublished	CP013032	Class D;OXA-61-like;Natural in Campylobacter coli;Alternative name CJ0299;
blaOXA-450	99.8708010336	774/774	1..774	NODE_3_length_154164_cov_96.482961	33734..34507	unknown beta-lactam	unpublished	KR061502	Class D;OXA-61-like;Natural in Campylobacter coli;;
blaOXA-452	99.8708010336	774/774	1..774	NODE_3_length_154164_cov_96.482961	33734..34507	unknown beta-lactam	unpublished	KR061505	Class D;OXA-61-like;Natural in Campylobacter jejuni;;
blaOXA-451	99.8708010336	774/774	1..774	NODE_3_length_154164_cov_96.482961	33734..34507	unknown beta-lactam	unpublished	KR061504	Class D;OXA-61-like;Natural in Campylobacter jejuni;;
blaOXA-61	99.8708010336	774/774	1..774	NODE_3_length_154164_cov_96.482961	33734..34507	amoxicillin,amoxicillin+clavulanic acid,ampicillin,ampicillin+clavulanic acid	15917560	AY587956	Class D;OXA-61-like;Natural in Campylobacter coli;;
blaOXA-489	99.8708010336	774/774	1..774	NODE_3_length_154164_cov_96.482961	33734..34507	unknown beta-lactam	unpublished	CP013733	Class D;OXA-61-like;Natural in Campylobacter coli;;
blaOXA-453	99.8708010336	774/774	1..774	NODE_3_length_154164_cov_96.482961	33734..34507	unknown beta-lactam	unpublished	KR061507	Class D;OXA-61-like;Natural in Campylobacter jejuni;;

Campylobacter TRING1C-1 – point mutations example

Table 14. Point mutations found in sequence TRING1C-1, Green – ResFinder, Blue – AMRFinder, Yellow – Different databases.

Lab #	RefRes	R01	R05	R06	R07	R09	R16	R17	R19	R20	R21	R23	R27	RefAMR	R24	R08	R11	R12	R13 *	R22	R28
	ResFinder													AMRF		Mix					
ResFinder (PointFinder)																					
AMRFinderPlus																					
23S						X	X														
gyrA T86I	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X

* used an in-house database

Table S 1. Unique point mutations reported by participant R09 in *Campylobacter* sequence TRING1C-1

Laboratory	Gene	Point mutations reported
R09	*gyrA*	R285K agg -> aag
	23S	327G>A g -> a, 643G>R g -> r, 554A>C a -> c, 298G>A g -> a, 571T>G t -> g, 1027A>G

Quinolone				
Mutation	**Nucleotide change**	**Amino acid change**	**Phenotype**	**PMID**
gyrA:p.T86I	aca -> ata	t -> i	nalidixic acid,ciprofloxacin	11266291, unpublished, 8384814, 16713726

TRING1C-1_Campylobacter.fasta

No class defined					
Mutation	**Nucleotide change**	**Amino acid change**	**Phenotype**	**PMID**	**Notes**
23S:g.571T>G	t -> g	-	Unknown phenotype	-	Phenotype not found in database
23S:g.298G>A	g -> a	-	Unknown phenotype	-	Phenotype not found in database
23S:g.364G>C	g -> c	-	Unknown phenotype	-	Phenotype not found in database
23S:g.327G>A	g -> a	-	Unknown phenotype	-	Phenotype not found in database
23S:g.1027A>G	a -> g	-	Unknown phenotype	-	Phenotype not found in database
gyrA:p.R285K	agg -> aag	r -> k	Unknown phenotype	-	Phenotype not found in database
23S:g.554A>C	a -> c	-	Unknown phenotype	-	Phenotype not found in database
23S:g.296C>G	c -> g	-	Unknown phenotype	-	Phenotype not found in database
23S:g.1752T>C	t -> c	-	Unknown phenotype	-	Phenotype not found in database

Campylobacter TRING1C-2 – database example

Table 15. Genes found in sequence TRING1C-2, Green – ResFinder, Blue – AMRFinder + ResFinder, Yellow – CARD and other databases.

Lab #	RefRes	R05	R07	R09	R16	R17	R19	R20	R21	R23	R27	RefAMR	R08	R11	R22	R28	R06	R13 *	R01	R12	R24
	ResFinder											ResAMR					Mix				
ResFinder																					
AMRFinderPlus																					
CARD																					
blaOXA												X			X						
blaOXA-193	X					X	X	X	X	X	X		X			X					
blaOXA-461																		X			
OXA-660																					X
tet(O)				X								X	X					X			X
tet(O/32/O)	X	X	X	X	X	X	X	X	X	X	X			X	X	X	X			X	X
tet(O/M/O)																		X			

* used an in-house database

assemblies

reads

- Difference in reference datasets in *blaOXA* gene reporting due to differences between databases
  - *blaOXA* in AMRFinderPlus (imperfect match to sequences of, f. ex. *blaOXA-193*) – potentially novel

Campylobacter TRING1C-2 – point mutation example
Not always a clear picture

Table 16. Point mutations found in sequence TRING1C-2, Green – ResFinder, Blue – AMRFinder, Yellow – Different databases.

Lab #	RefRes	R01	R05	R06	R07	R09	R16	R17	R19	R20	R21	R23	R27	RefAMR	R24	R08	R11	R12	R13 *	R22	R28
	ResFinder													AMRF		Mix					
ResFinder (PointFinder)																					
AMRFinderPlus																					
23S						X	X		X												
50S L22 A103V		X												X	X		X	X		X	X
cmeR						X	X		X												
gyrA							X		X												
gyrA 2 T86I				X																	
gyrA T86I	X					X		X		X	X	X	X	X	X	X	X	X	X	X	X

* used an in-house database

- 50S L22 A103V mutation - equally common among resistant and sensitive isolates in a set of 516 Campylobacter isolates (Dahl et al. 2021)
- Point mutation free text reporting

Campylobacter TRING1C-4 – nomenclature example

Table 19. Genes found in sequence TRING1C-4, Green – ResFinder, Blue – AMRFinder + ResFinder, Yellow – CARD and other databases.

Lab #	RefRes	R05	R07	R09	R16	R17	R19	R20	R21	R23	R27	RefAMR	R08	R11	R22	R28	R06	R13 *	R01	R12	R24
	ResFinder											ResAMR					Mix				
ResFinder																					
AMRFinderPlus																					
CARD																					
aad9												X			X	X					X
aadE												X	X		X	X			X		X
ant(6)-Ia	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	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
tet(O)		X		X								X			X	X	X	X			X
tet(O/32/O)	X		X							X											
tet(O/M/O)																		X			

* used an in-house database

nomenclature

Best out of 3?
"Average"?
All?

- Genes of aminoglycoside nucleotidyltransferase subfamily (ANT(6)-I) are also known as aminoglycoside adenyltransferases of the AADE family (Hormeño et al., 2018) - **difference in nomenclature between databases**

Conclusions and lessons learned

Overall, the expected targets were identified by participants, but some fine-tuning is needed

Small differences were observed due to:

- Different input data types (reads vs assemblies)
- Different tools and databases
- Differences in nomenclature in different databases
- Genes in difficult genomic regions can be potentially missed during assembly
- Identification of the exact variant through mapping might be difficult if many closely related variants in the database are present

In general:

Keep your database updated at all times

Decide on a strategy when using more than one databases:

- If you merge the output from different databases – be critical and assess the genes individually – more time consuming
- Use one well curated database and a tool with a clear output

To be expected in the next round:

- More detailed and clearly formulated questions will help with result analysis
- Completely new reporting system – any suggestions that would make it easier for you..?
- EQA and RingTrial coordinated

Thank you for your attention!

Contact us at **fwdamr@ssi.dk**