FWD AMR· RefLabCap

Lessons learned from EQA1-WGS-AMR and RingTrial1-WGS-AMR

Presented by

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on behalf of the Methods Team

Foodborne Infections (FBI)
Statens Serum Institut
SSI

DTU

STATENS SERUM INSTITUT

EQA and RingTrial in FWD AMR-RefLabCap

EQA1-WGS-AMR

Material to analyse:
Live bacteria
(DNA purification and WGS required)

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

Comparison of performance of the NRLs in AMR gene and point mutation detection **based on sequences produced by each NRL**
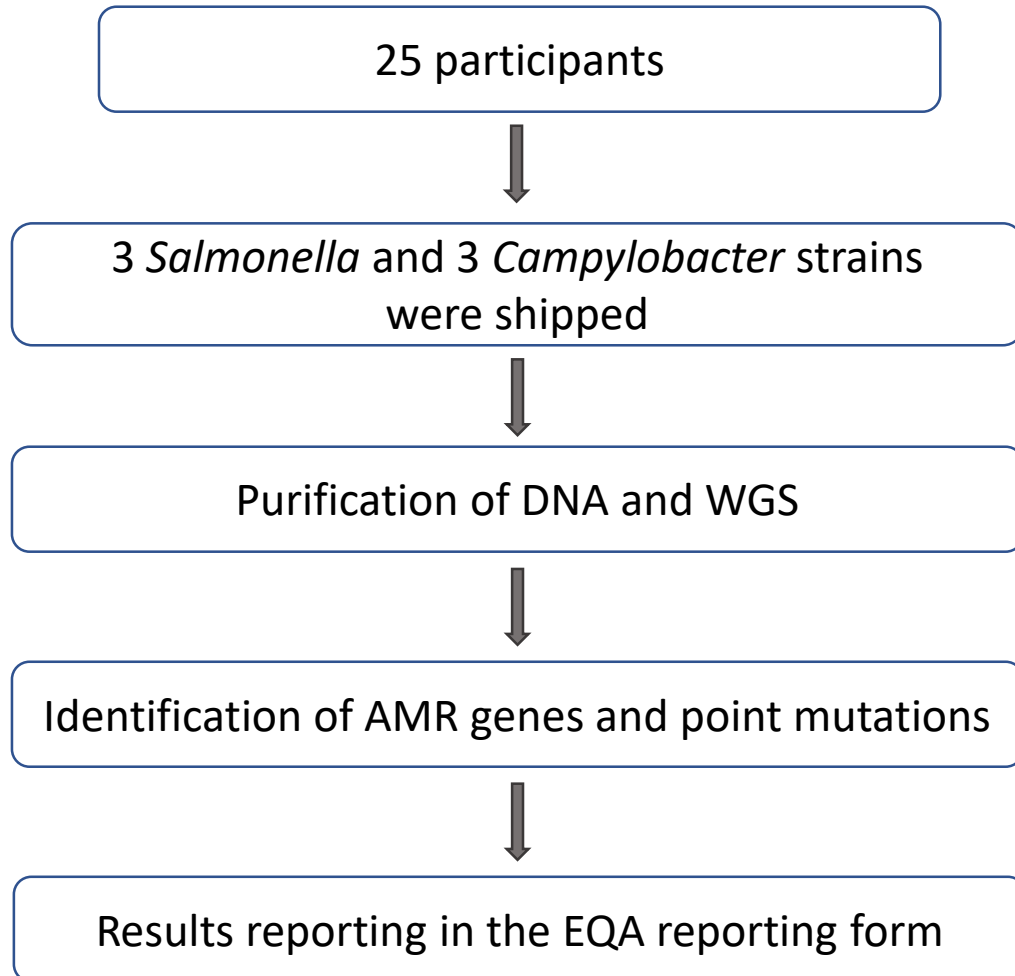
RingTrial1

Material to analyse:
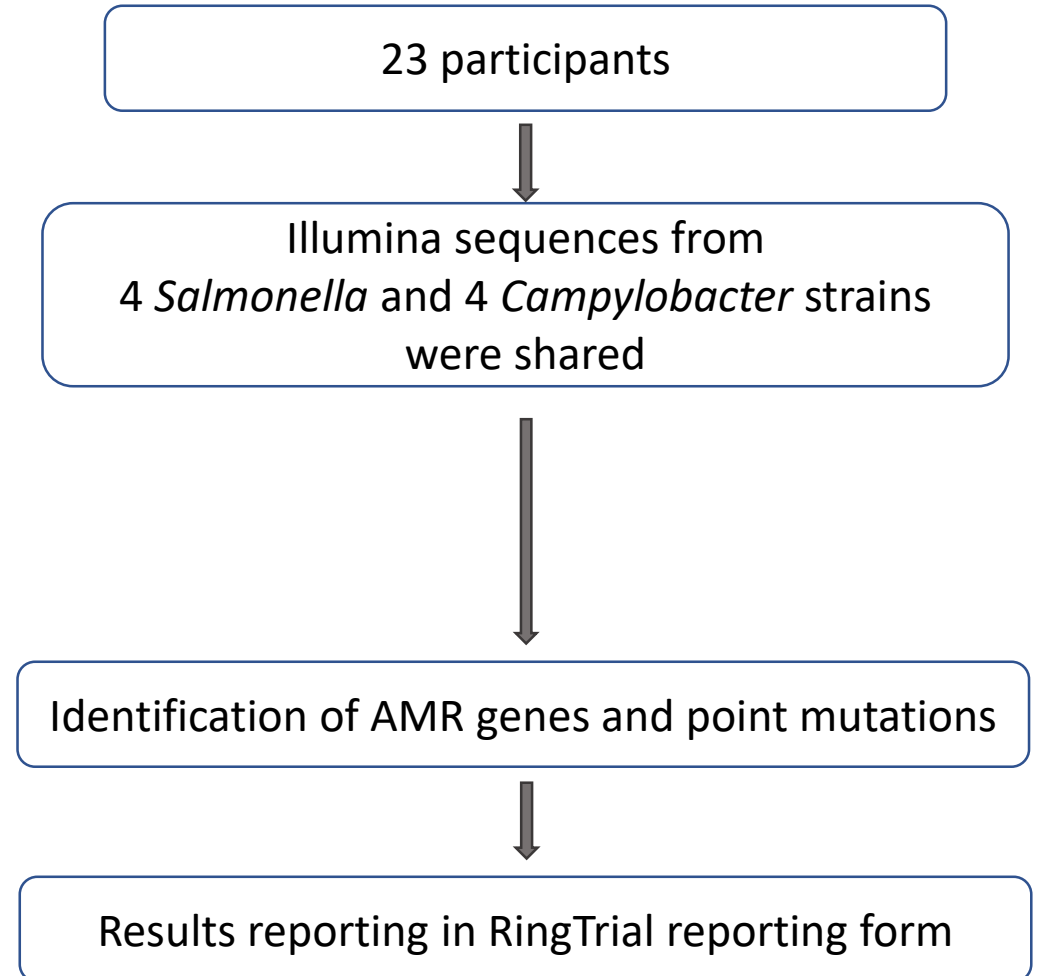Provided sequences
(reads or assemblies)

Comparison of the outcomes of different databases, tools and bioinformatic pipelines used by NRLs

Comparison of performance of the NRLs in AMR gene and point mutation detection **based on provided sequences**
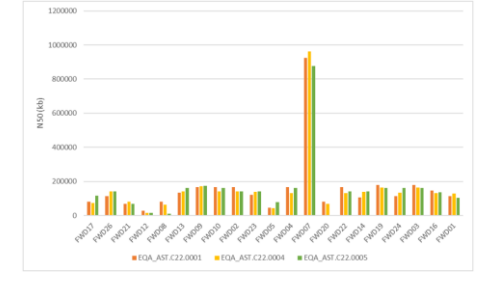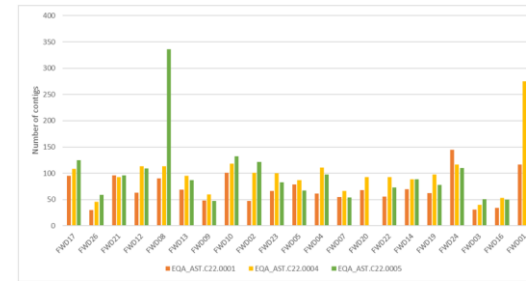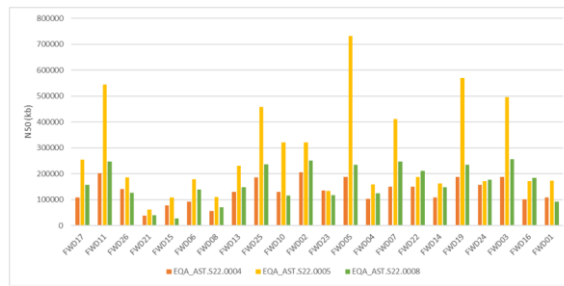
EQA1-WGS-AMR

RingTrial1-WGS-AMR

```
┌─────────────────────────┐        ┌─────────────────────────┐
│     25 participants     │        │     23 participants     │
└─────────────────────────┘        └─────────────────────────┘
            ↓                                   ↓
┌─────────────────────────┐        ┌─────────────────────────┐
│ 3 Salmonella and 3      │        │ Illumina sequences from │
│ Campylobacter strains   │        │ 4 Salmonella and 4      │
│ were shipped            │        │ Campylobacter strains   │
│                         │        │ were shared             │
└─────────────────────────┘        └─────────────────────────┘
            ↓                                   ↓
┌─────────────────────────┐                     │
│ Purification of DNA     │                     │
│ and WGS                 │                     │
└─────────────────────────┘                     │
            ↓                                   ↓
┌─────────────────────────┐        ┌─────────────────────────┐
│ Identification of AMR   │        │ Identification of AMR   │
│ genes and point         │        │ genes and point         │
│ mutations               │        │ mutations               │
└─────────────────────────┘        └─────────────────────────┘
            ↓                                   ↓
┌─────────────────────────┐        ┌─────────────────────────┐
│ Results reporting in    │        │ Results reporting in    │
│ the EQA reporting form  │        │ RingTrial reporting form│
└─────────────────────────┘        └─────────────────────────┘
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Quality of sequences produced in NRLs

Salmonella

Campylobacter
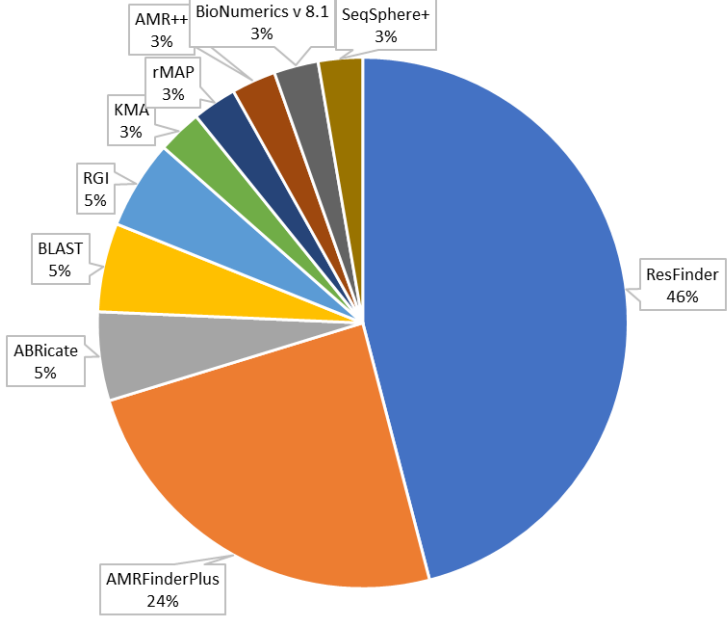
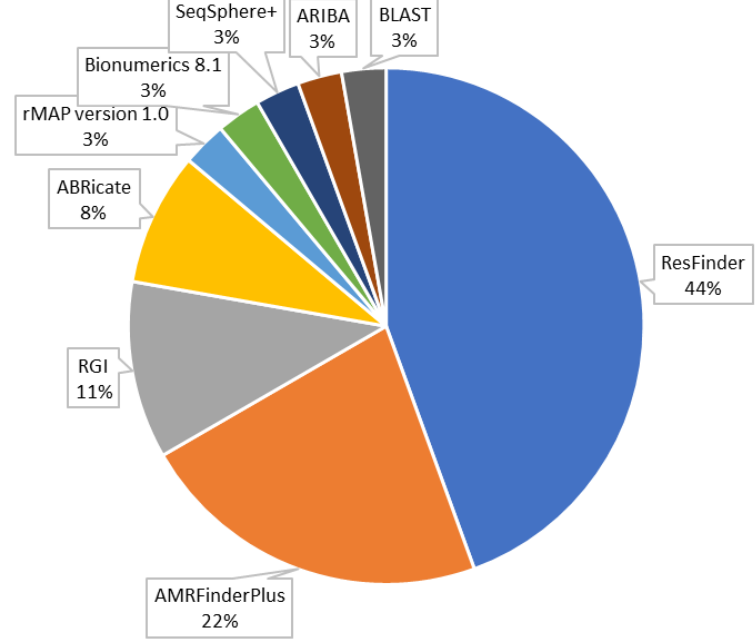**Salmonella gene detection tools EQA1-WGS-AMR**

- ResFinder 46%
- AMRFinderPlus 24%
- ABRicate 5%
- BLAST 5%
- RGI 5%
- KMA 3%
- rMAP 3%
- AMR++ 3%
- BioNumerics v 8.1 3%
- SeqSphere+ 3%

Campylobacter gene detection EQA1-WGS-AMR

- ResFinder 44%
- AMRFinderPlus 22%
- RGI 11%
- ABRicate 8%
- rMAP version 1.0 3%
- Bionumerics 8.1 3%
- SeqSphere+ 3%
- ARIBA 3%
- BLAST 3%

Tools

Salmonella point mutation detection tools EQA1-WGS-AMR

- ResFinder (PointFinder) 60%
- AMRFinderPlus 23%
- KMA 4%
- ARIBA 4%
- ABRicate 3%
- BioNumerics v 8.1 3%
- SeqSphere+ 3%
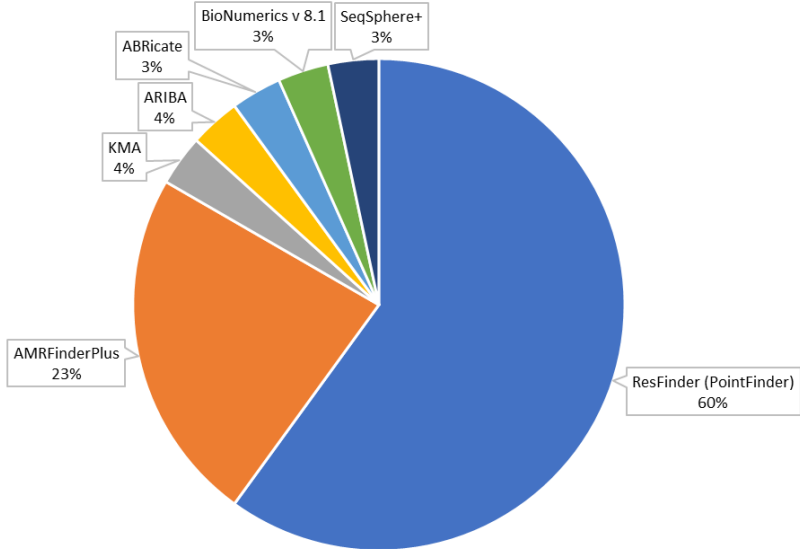
Campylobacter point mutation detection EQA1-WGS-AMR

- ResFinder (PointFinder) 59%
- AMRFinderPlus 22%
- KMA 3%
- ARIBA 4%
- ABRicate 4%
- Bionumerics 8.1 4%
- SeqSphere+ 4%

EQA1-WGS-AMR

RefLabCap Network Me...
Copenhage...
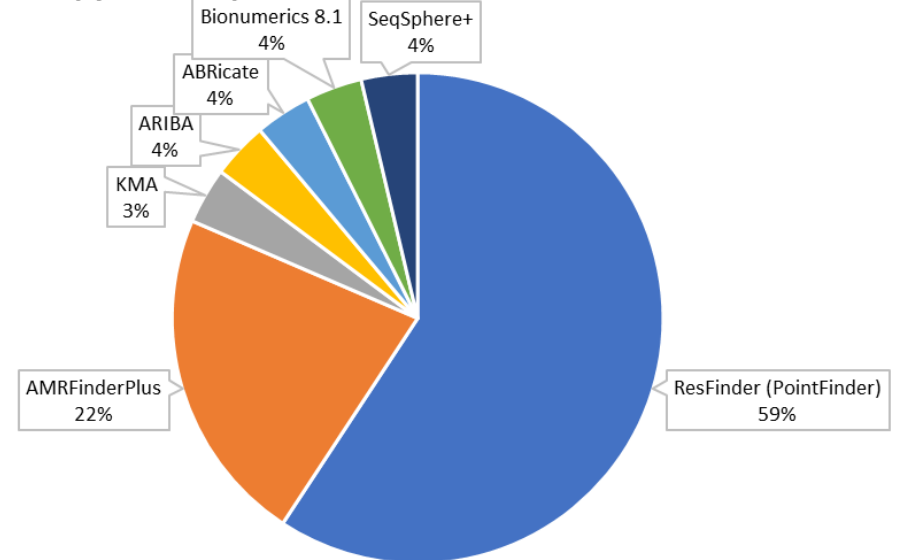
Gene identification in EQA - *Salmonella*

- Reference sets based on 2 databases
- No correct / incorrect evaluation
- Grouping based on tools used

Table 1

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		
blaTEM-1	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		
dfrA12	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		
fosA	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					
qacL										X													
qnrS1	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				
tet(M)	X	X	X		X	X			X	X	X	X	X		X								

- Challenges with nomenclature

Table 2

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	X
sul	X	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	X

Gene identification in EQA - *Campylobacter*

- Reference sets based on databases
- No correct / incorrect evaluation
- Grouping based on tools used

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

- Challenges with nomenclature

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

Gene identification in RT - *Salmonella*

ResFinder

ResFinder and AMRFinderPlus

CARD and other databases

variant

| 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
aadA16	X	X	X	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
aph(4)-Ia	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X
aph(6)-Id	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
arr-3	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
blaTEM-1B	X	X	X	X	X		X	X	X	X		X		X			X	X	X
catA2	X		X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X
dfrA27	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
qacE	X			X				X							X				
qacEdelta1												X		X	X			X	X
qnrS1						X													
sitABCD						X													
sul1	X	X	X		X		X	X	X	X	X	X	X	X	X	X	X	X	X
sul2	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X		X	
tet(A)						X													
tet(D)	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X

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

Gene identification in RT - *Campylobacter*

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
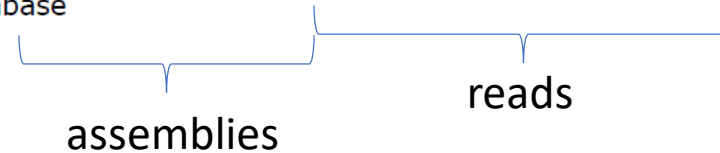
Campylobacter TRING1C-1 – blaOXA example

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;;

Point mutation identification in EQA

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				

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	

Gene variant added to ResFinder db in June 2022

*No data available due to failing in sequencing

Point mutation identification in RT

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	

Not present in AMRFinderPlus database (considered non-informative)

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

Lessons learned

- Slightly different results might be obtained depending on whether reads or assemblies are used

- Different tools and databases can also produce different results

- Keep in mind the nomenclature differences between databases

- Make sure your database is updated, especially if you use a local database

- When using output from more than one database / tool, curate the genes / point mutations obtained – requires more time and effort
- Otherwise – choose one well curated database

Upcoming 2nd round of EQA and RT

EQA2-WGS-AMR

34 registered participants

8 May 2023 – shipment of DNA from SSI
16 June 2023 – online submission deadline

RingTrial2-WGS-AMR

39 registered participants

15 May 2023 – sequences and assemblies available for download
16 June 2023 – online submission deadline

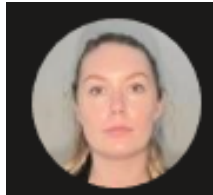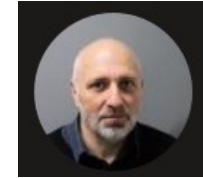- More detailed and clearly formulated questions will help with the result analysis
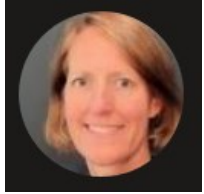
Eva Litrup
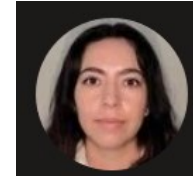
Karen Loaiza Conza

Eva Møller Nielsen

Thank you for your attention and thank you to these people!

Jeppe Boel

Anne Sophie Majgaard Uldall

Laboratory personnel (TBL)

Colleagues from DTU