FWD AMR· RefLabCap

STATENS SERUM INSTITUT

DTU

Highlights from EQA3-WGS-AMR and RingTrial3-WGS-AMR

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on behalf of EQA team

Foodborne Infections (FBI)
Statens Serum Institut

Outline

EQA3 and RingTrial3 differences

EQA:

- DNA sequencing quality
- DNA and sequence evaluation results
- Reporting genes and point mutations (PMs)
- QC effect on the gene and PM results

RingTrial:

- Methods
- Reporting genes and point mutations (PMs)

Conclusions from EQA3 and RT3

Three years of EQA and RT

EQA3-WGS-AMR

30 participants

DNA from 3 *Salmonella* and 3 *Campylobacter* isolates was distributed

Whole Genome Sequencing

Identification of AMR genes and point mutations according to the provided EQA3 protocol

Results reporting in the EQA reporting form

RingTrial3-WGS-AMR

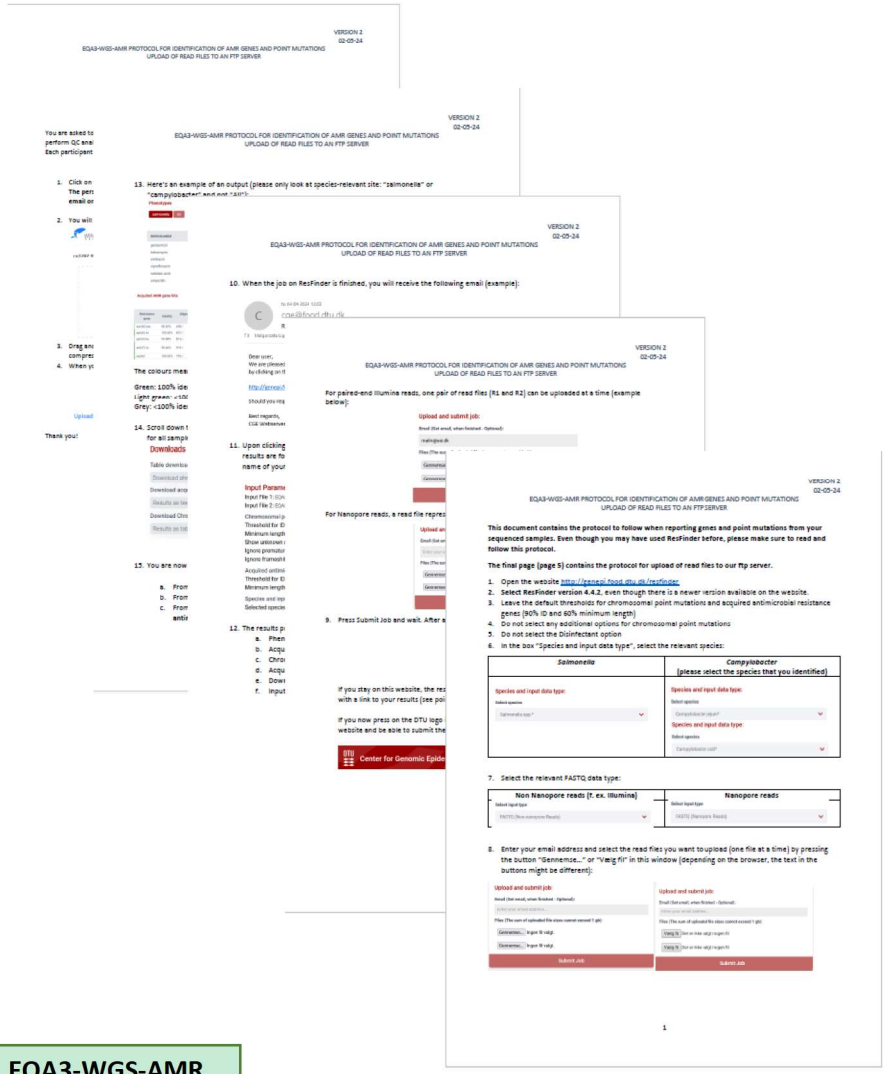
32 participants

Illumina sequences from 4 *Salmonella* and 4 *Campylobacter* isolates were shared

Identification of AMR genes and point mutations using the usual pipelines

Results reporting in RingTrial reporting form

EQA3 protocol



EQA3-WGS-AMR

Type of input file (fastq)

One version of online tool (4.4.2)

Cut-off (default: 90% identity and 60% min. length)

Reporting genes and PMs from the same files, not from the website

Sequencing quality the only variable factor – enabling to evaluate its effect on gene and PM reporting

EQA: DNA and sequencing quality

EQA3-WGS-AMR

EQA3-WGS-AMR

DNA evaluation results

Information provided by participants:

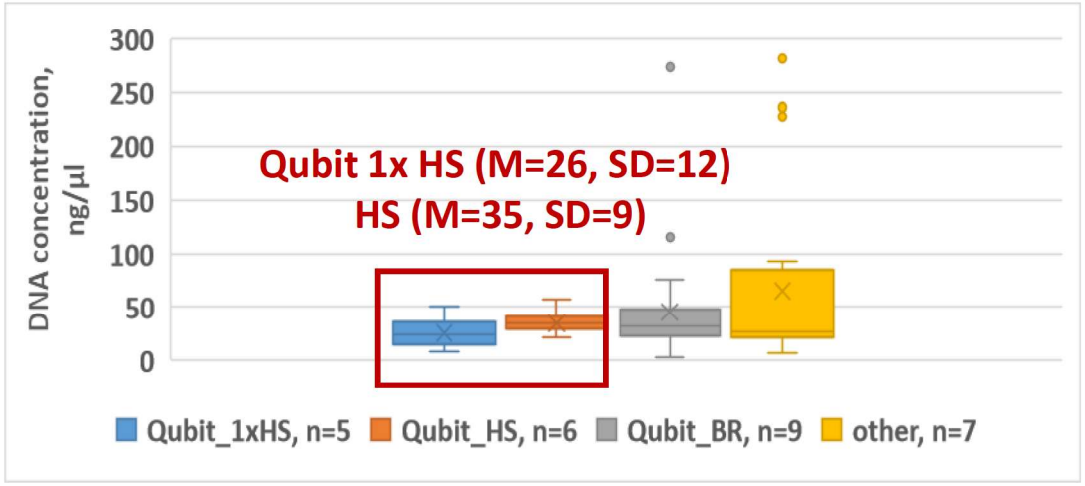
- concentration, ng/µl
- method/instrument
- kit
- other details, e.g. volume

NEW

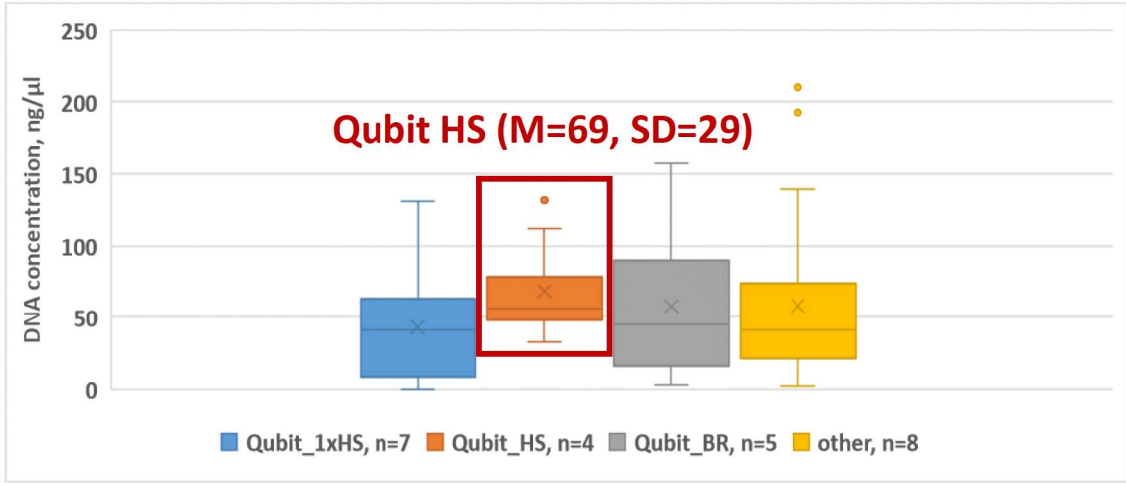
Qubit was most widely used method, 73% (S) and 66% (C)

For Salmonella, the use of Qubit 1xHS and HS kit resulted in less variable DNA concentrations compared to other Qubit kits

Salmonella, n=26



Campylobacter, n=23



Sequence QC evaluation

Information provided by participants:

NEW

- sequencing technology
- library preparation kit
- reads as *.fastq* files

We evaluated the reads using SSI pipelines:

• No. of reads, thousands	no threshold
• Contamination	<5% of species 2
• Av. coverage	>30X
• Genome length at 25X	4.4 Mb - 5.8 Mb (S), 1.5 Mb - 1.9 Mb (C)
• No. of contigs at 25X	<500
• No. of contigs at <25X	<1000
• N50, Kbp	>30

Sequence evaluation results - overview

Information field	*Salmonella*	*Campylobacter*
Technology		
Illumina	23	22
Ion Torrent	2	2
Nanopore	1	1
Passed QC for all strains	21 (80%)	17 (70%)
QC did not pass for 1 strain	E06, E33, E37	E05, E20, E24, E35
QC did not pass for 2 strains	E35	E36, E37
QC did not pass for 3 strains	E05	E21, E33,

Most often only N50 did not pass the threshold of >30 Kbp, often associated with:

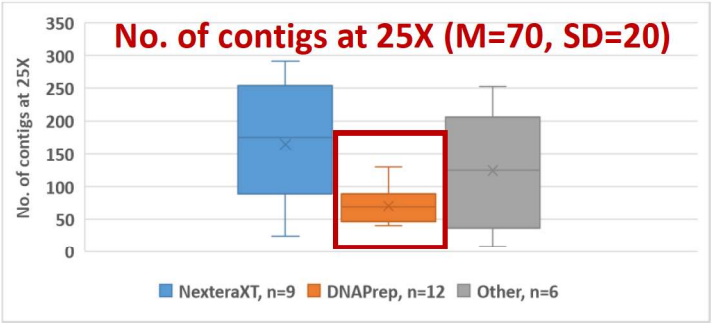
- higher number of contigs, though still of <500
- av. coverage between 35X to 150X

Sequence coverage of <30X, and consequently genome length of < 4.4 Mb - 5.8 Mb were the most common reason for not passing the QC

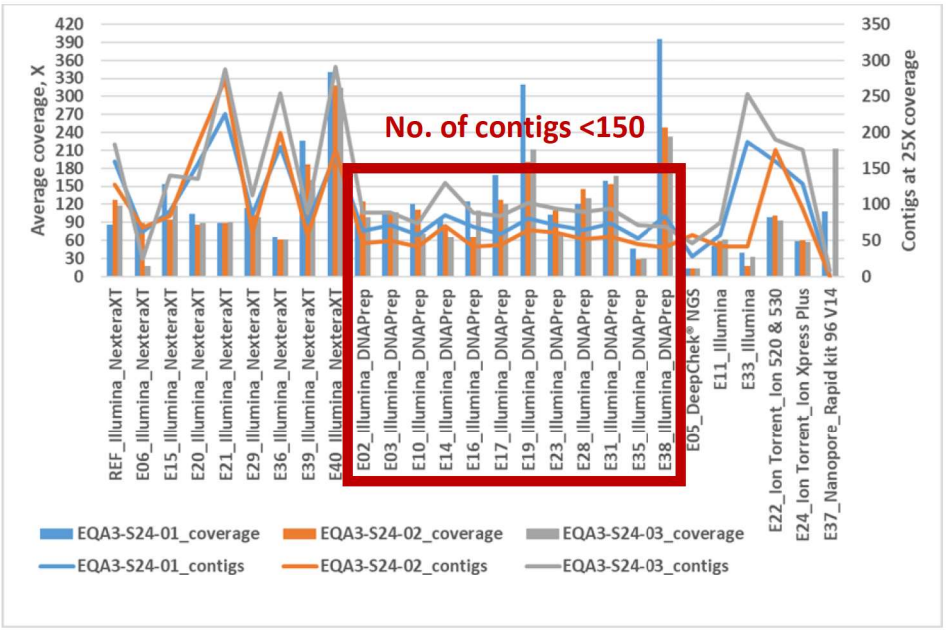
EQA3-WGS-AMR

Sequence evaluation results – no. of contigs vs library preparation kits

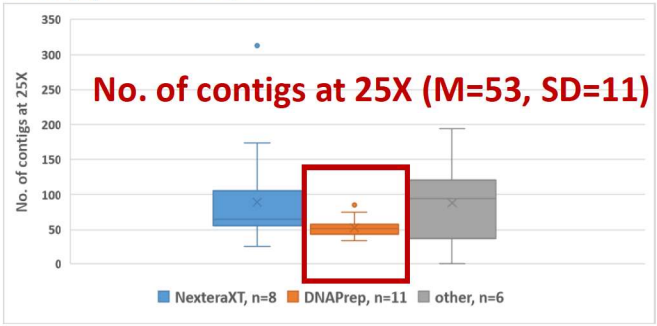
Salmonella, n=26

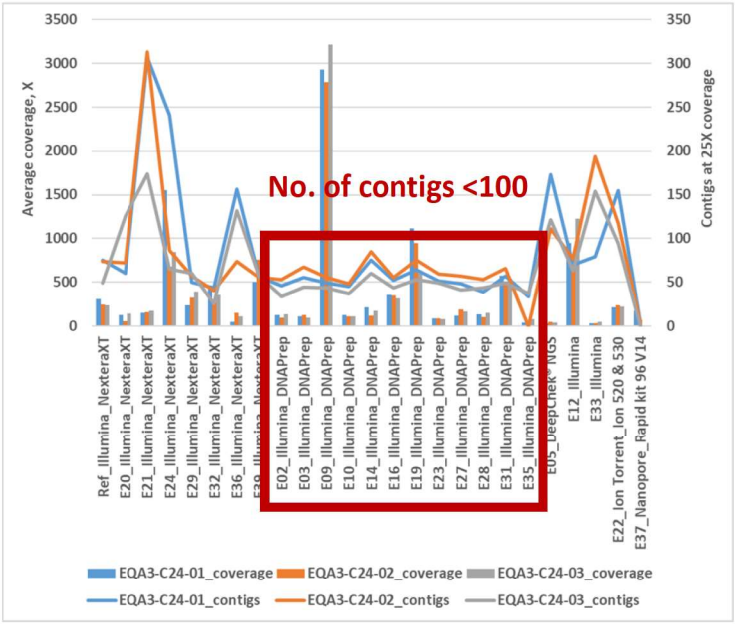


For both species, DNA Prep provided fewer contigs and less variation in number of contigs



Campylobacter, n=24





Reporting genes and point mutations

EQA3-WGS-AMR

Salmonella genes

87% of genes in all samples reported by more than 90% participants

Examples of not following the protocol:

- Reporting additional genes (detected only when contigs were used)
- Not reporting genes due to identity and coverage cut-offs lower than 100% (90% and 60% in the EQA3 protocol)
- Reporting a variant with a higher identity and coverage instead of both
- Reporting one of the two alternative names, even though both are listed

EQA3-S24-01

	Ref	E02	E03	E05	E06	E10	E11	E14	E15	E16	E17	E19	E20	E21	E22	E23	E24	E28	E29	E31	E33	E35	E36	E37	E38	E39	E40	%C
aac(3)-IIa	X		X	X		X		X	X	X	X	X	X	X	X	X	X	X	X	X		X	X		X		X	77
aac(6')-Iaa	X	X	X	X	X	X		X	X	X	X	X		X	X	X	X	X	X	X		X	X	X	X	X	X	88
aph(3'')-Ib	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X		X	92
aph(6)-Id	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X	X	X		X	X	X	92
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	X	X	X	X	100
floR	X	X	X	●	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	96
qnrS1	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	100
sul2	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	100
tet(A)	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	100

EQA3-S24-02

	Ref	E02	E03	E05	E06	E10	E11	E14	E15	E16	E17	E19	E20	E21	E22	E23	E24	E28	E29	E31	E33	E35	E36	E37	E38	E39	E40	%C
aac(6')-Iaa	X	X	X	X	X	X		X	X	X	X	X		X	X	X	X	X	X	X	X	X	X		X	X	X	92
aadA1	X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X		X		X	92
aadA2	X	X	X	●	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X		X	X	X	92
ant(3'')-Ia	X							X		X		X		X				X				X					X	28
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		X	X	X	100
blaTEM-1B	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X	100
cmlA1	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X	100
dfrA12	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X	100
floR	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X	100
fosA7	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X	100
qnrS1	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X	100
sul2	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X	100
sul3	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X		X	96

EQA3-S24-03

	Ref	E02	E03	E05	E06	E10	E11	E14	E15	E16	E17	E19	E20	E21	E22	E23	E24	E28	E29	E31	E33	E35	E36	E37	E38	E39	E40	%C
aac(6')-Ib	X			●															X	X					X			12
aac(6')-Ib3	X	X	X		X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X		X	88
aac(6')-IIc	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X	100
aac(6')-Iaa	X	X	X	X	X	X		X	X	X	X	X		X	X	X	X	X	X	X	X	X	X		X	X	X	92
aadA2	X	X	X	●	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X	96
aph(3')-Ia	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X	100
aph(3'')-Ib	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X		X	96
aph(6)-Id	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X		X	96
blaSHV-12	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X	100
blaTEM-1B	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X	100
dfrA19	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X	100
ere(A)	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X		X	X	X	96
qnrB2	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X	100
sul1	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X	100
sul2	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X		X	96
tet(B)	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X	100
tet(D)	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X	100

Salmonella PMs

EQA3-S24-01																										
	Ref	E02	E03	E05	E06	E10	E11	E14	E15	E16	E17	E19	E20	E21	E22	E23	E24	E28	E29	E31	E33	E35	E36	E37	E38	E39	E40	%C
parC T57S	X	X	X	X	X	X	●	X	X	X	X	●	X	X	X	X	X	X	X	X	X	X	X		●	X	X	88
gyrA D87N	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X	100
EQA3-S24-02																												
	Ref	E02	E03	E05	E06	E10	E11	E14	E15	E16	E17	E19	E20	E21	E22	E23	E24	E28	E29	E31	E33	E35	E36	E37	E38	E39	E40	%C
parC T57S	X	X	X	X	X	X	●	X	X	X	X	X	●	X	X	X	X	X	X	X	X	X	X		●	X	X	88

EQA3-WGS-AMR

Salmonella
Predicted phenotypes

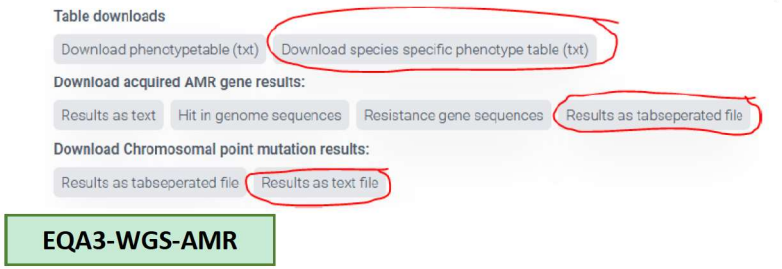
EQA3-S24-01

	Ref
Amikacin	NWT
Ampicillin	NWT
Azithromycin	WT
Cefotaxime	NWT
Ceftazidime	NWT
Chloramphenicol	NWT
Ciprofloxacin	NWT
Colistin	WT
Gentamicin	NWT
Meropenem	WT
Nalidixic acid	NWT
Sulfamethoxazole	NWT
Tetracycline	NWT
Tigecycline	WT
Trimethoprim	WT

EQA3-S24-02

	Ref
Amikacin	NWT
Ampicillin	NWT
Azithromycin	WT
Cefotaxime	NWT
Ceftazidime	NWT
Chloramphenicol	NWT
Ciprofloxacin	NWT
Colistin	WT
Gentamicin	WT
Meropenem	WT
Nalidixic acid	WT
Sulfamethoxazole	NWT
Tetracycline	WT
Tigecycline	WT
Trimethoprim	NWT

Phenotypes for antimicrobials included in the predicted phenotype question in the reporting scheme



EQA3-S24-03

	Ref
Amikacin	NWT
Ampicillin	NWT
Azithromycin	WT
Cefotaxime	NWT
Ceftazidime	NWT
Chloramphenicol	WT
Ciprofloxacin	NWT
Colistin	WT
Gentamicin	NWT
Meropenem	WT
Nalidixic acid	WT
Sulfamethoxazole	NWT
Tetracycline	NWT
Tigecycline	WT
Trimethoprim	NWT

Campylobacter genes

57% of genes in all samples reported by more than 90% participants

<u>Examples of not following the protocol:</u>

- Not reporting genes with identity lower than 100% (EQA3 protocol: above 90%)
- Reporting genes detected in contigs (assemblies instead of reads)
- Reporting genes detected using other tools and databases (AMRFinderPlus and CARD)

EQA3-C24-01																									
	Ref	E02	E03	E05	E09	E10	E12	E14	E16	E19	E20	E21	E22	E23	E24	E27	E28	E29	E31	E32	E33	E35	E36	E37	E39	%C
aadE-Cc	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	96
blaOXA-489	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	96
tet(O)	X		X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X	X	88
EQA3-C24-02																									
	Ref	E02	E03	E05	E09	E10	E12	E14	E16	E19	E20	E21	E22	E23	E24	E27	E28	E29	E31	E32	E33	E35	E36	E37	E39	%C
ant(6)-Ia	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X	100
aph(3')-III	X	X	X	X	X	X		X	X	X		X	X		X	X	X	X	X	X			X		X	78
blaOXA-193	X	X	X			X		X	X	X	X	X	X	X	X	X	X	X	X	X			X		X	78
cat(pC194)	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X		X	96
erm(B)	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X	100
tet(O/32/O)	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X		96
EQA3-C24-03																									
	Ref	E02	E03	E05	E09	E10	E12	E14	E16	E19	E20	E21	E22	E23	E24	E27	E28	E29	E31	E32	E33	E35	E36	E37	E39	%C
aph(2'')-If	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	96
aph(3')-III	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X			88
blaOXA-193	X	X	X			X	X	X	X	X	X	X		X	X	X	X	X	X	X		X	X		X	79
cat	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	96
tet(O)	X	X	X		X	X	X	X	X	X			X	X	X	X	X	X	X	X	X	X	X	X	X	88

EQA3-WGS-AMR

Campylobacter PMs

Query sequence less than 100% identical than the reference query sequence

Mutation listed as "undefined"

	Ref	E02	E03	E05	E09	E10	E12	E14	E16	E19	E20	E21	E22	E23	E24	E27	E28	E29	E31	E32	E33	E35	E36	E37	E39	%C
EQA3-C24-01																										
23S A2075G	X	X	X	●	X	X	X	X	X	X	X	X	X	X	X	X	X	X	●	X	X	X	X		X	91
gyrA T86I	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	96
EQA3-C24-02																										
	Ref	E02	E03	E05	E09	E10	E12	E14	E16	E19	E20	E21	E22	E23	E24	E27	E28	E29	E31	E32	E33	E35	E36	E37	E39	%C
gyrA T86I	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X		X	95
rpsL K43R	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X		X	100
EQA3-C24-03																										
	Ref	E02	E03	E05	E09	E10	E12	E14	E16	E19	E20	E21	E22	E23	E24	E27	E28	E29	E31	E32	E33	E35	E36	E37	E39	%C
gyrA T86I	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	100

Campylobacter predicted phenotypes

EQA3-C24-01

	Ref
Ampicillin	WT
Chloramphenicol	ND
Ciprofloxacin	NWT
Erythromycin	NWT
Gentamicin	WT
Tetracycline	NWT

EQA3-C24-02

	Ref
Ampicillin	WT
Chloramphenicol	ND
Ciprofloxacin	NWT
Erythromycin	NWT
Gentamicin	WT
Tetracycline	NWT

EQA3-C24-03

	Ref
Ampicillin	WT
Chloramphenicol	ND
Ciprofloxacin	NWT
Erythromycin	WT
Gentamicin	NWT
Tetracycline	NWT

# Antimicrobial	Class	WGS-predicted phenotype	Match	Genetic background
erythromycin	macrolide	Resistant	3	
ciprofloxacin	quinolone	Resistant	3	
ampicillin	beta-lactam	No resistance	0	
tetracycline	tetracycline	Resistant	2	tet(O) (tet(O)_M20925), tet(O) (tet(O)_Y07780)
streptomycin	aminoglycoside	Resistant	3	aadE-Cc (aadE-Cc_CP013733)
gentamicin	aminoglycoside	No resistance	0	

EQA3-WGS-AMR

Sequence quality effect on the gene and point mutations results

EQA3-WGS-AMR

Overall evaluation of AMR determinant detection and reporting

Participants' ability to correctly detect and report *Salmonella* and *Campylobacter* genes and PMs

Good quality sequences for all samples and following the protocol

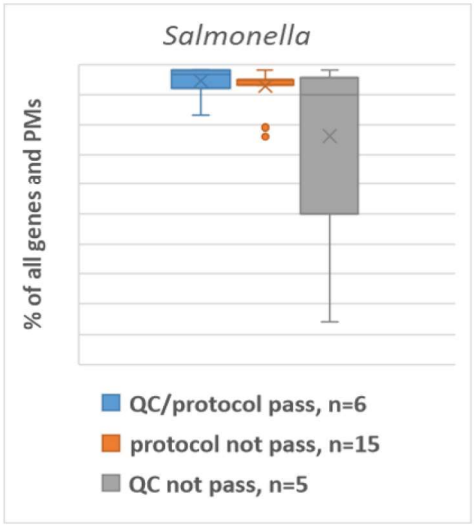
- *Salmonella*, M=95%, SD=6%
- *Campylobacter*, M=96%, SD=6%

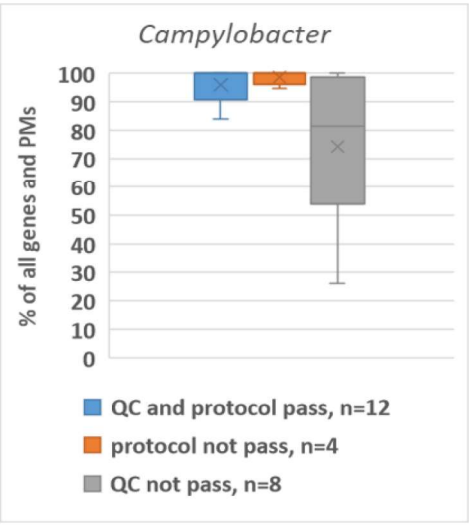
High quality sequencing for all samples, but not following the protocol

- *Salmonella*, M=93%, SD=6%;
- *Campylobacter*, M=99%, SD=3%

Not passing sequencing QC for at least one sample, and followed the protocol

- *Salmonella*, M=76%, SD=35%
- *Campylobacter*, M=74%, SD=27%





Ring-trial methods

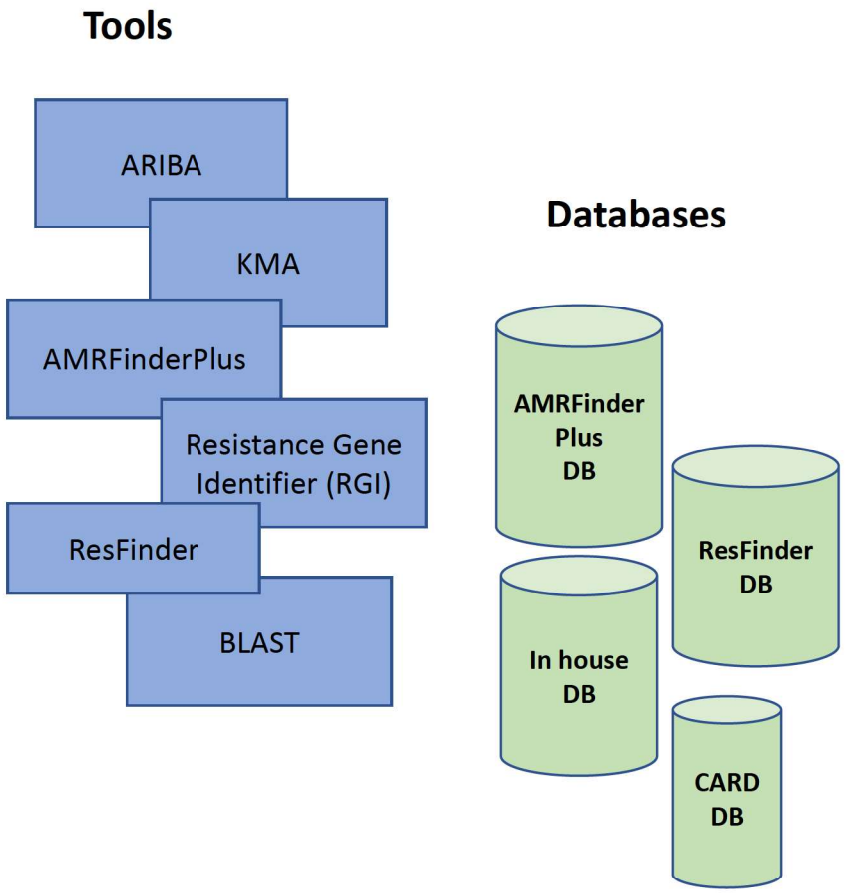
RingTrial3-WGS-AMR

Methods used for gene and point mutation detection and reporting

- In the survey we asked about:
  - ✓Tools and input
  - ✓Databases
  - ✓Thresholds for sequence length and identity
  - ✓Result reporting strategy

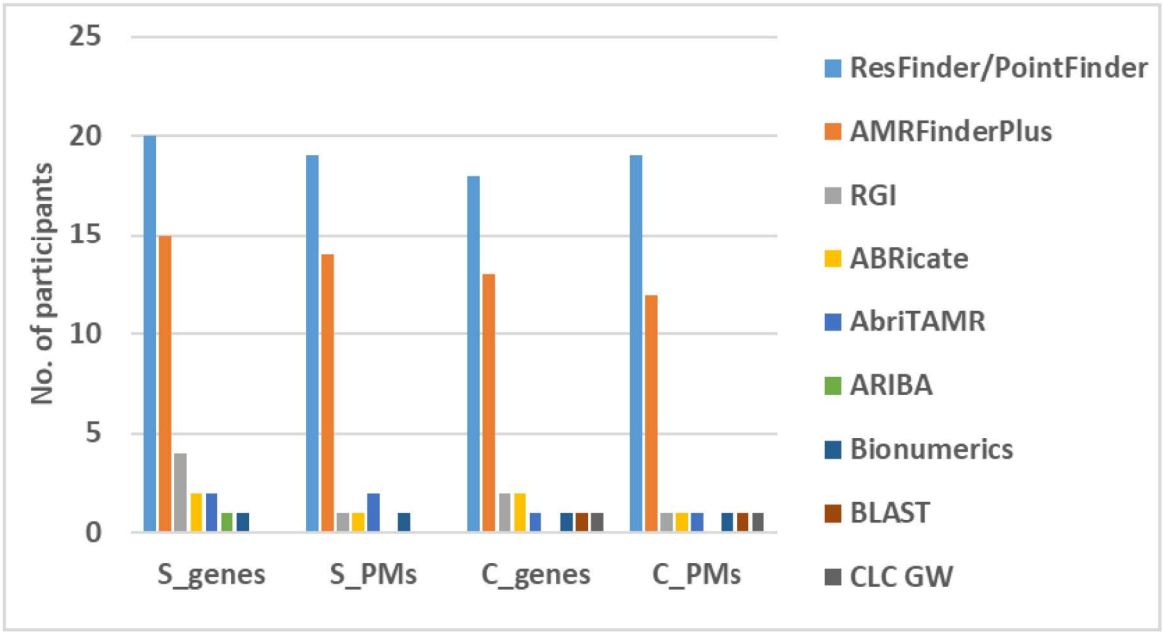
Version

NEW

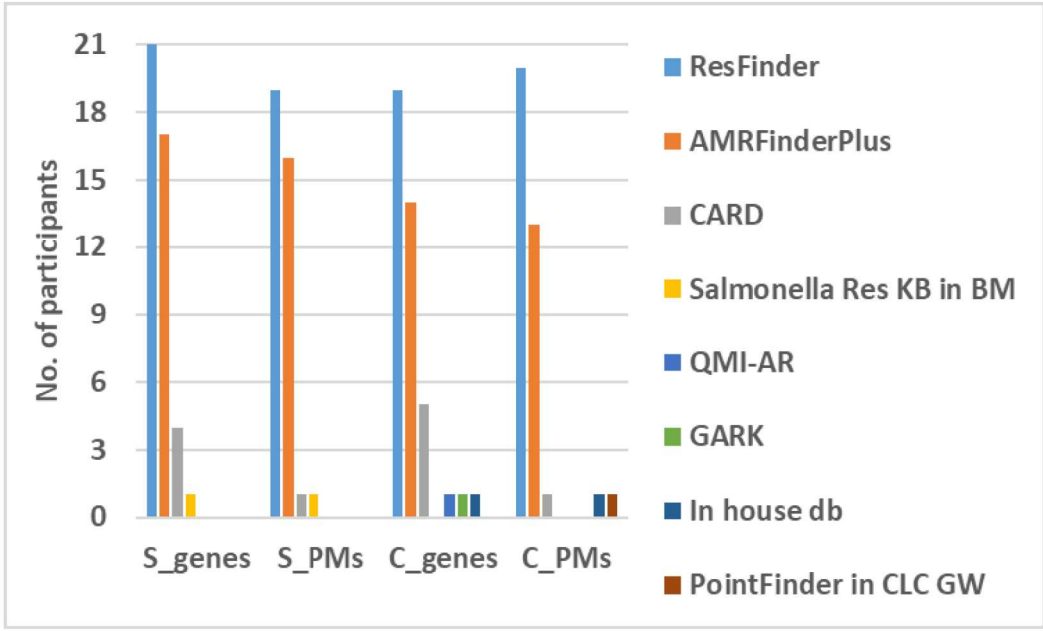


Tools and databases used

Tools



Databases



ResFinder (PointFinder) and AMRFinder were the tools and databases used most commonly, in combination or alone

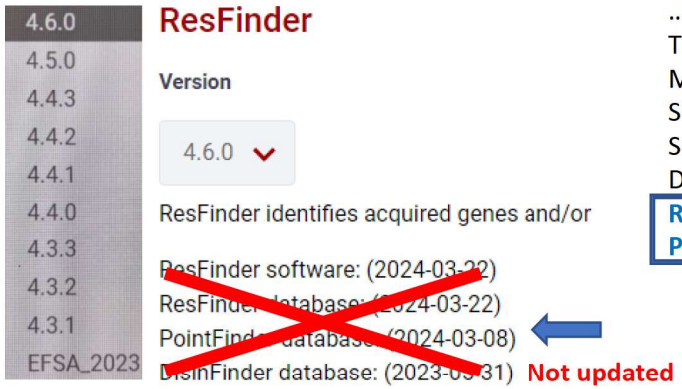
Version reporting for tools and databases

Unique combination	Lab ID[A]	Tool_version_input[B]	Database_version	Identity (%)	Coverage (%)
		1 tool	1 database		
1	R07[C]	ResFinder_4.5.0_N	ResFinder_4.5.0	90	60
1	R16	ResFinder_4.5.0_N	ResFinder_4.5.0	90	60
2	R15[D]	ResFinder_4.5.0_N_R	ResFinder_4.5.0	90	60
3	R17	ResFinder_4.5.0_R	ResFinder_4.5.0	90	60
4	R30	ResFinder_4.5.0_R	ResFinder_4.5.0	90	60
5	R25	ResFinder_4.5.0_N	ResFinder_2.3.1	90	60
6	R33	ResFinder_4.0_R	ResFinder_4.0	90	60
7	R23	ResFinder_2.2.1_R	ResFinder_2.2.1	80	60
8	R01	AMRFinderPlus_3.11.2_N	AMRFinderPlus_2022-12-19.1	100	90
9	R04	AMRFinderPlus_3.12.8_N	AMRFinderPlus_2024-05-02.2	90	60

CGE users

http://genepi.food.dtu.dk/resfinder

Available tool/software versions



Input Parameters
...
Threshold for ID: 90.0 %
Minimum length: 60.0 %
Species and input data type
Selected species: Salmonella
Database versions
ResFinder-2.4.0
PointFinder-4.1.1

Database versions are available in the ResFinder output online

Versions available for download

https://bitbucket.org/genomicepidemiology/resfinder_db/downloads/?tab=tags

Downloads | Tags | Branches

Tag	Commit	Date	Download
2.4.0 **Database**	d1e607b	2024-08-06	zip · gz · bz2
resfinder-4.6.0 **Software**	d1e607b	2024-08-06	zip · gz · bz2
2.3.1	c8c38c3	2024-03-22	zip · gz · bz2
resfinder-4.5.0	c8c38c3	2024-03-22	zip · gz · bz2
2.3.0	16d7fd3	2024-03-08	zip · gz · bz2
resfinder-4.4.3	16d7fd3	2024-03-08	zip · gz · bz2
2.2.1	208efbd	2023-10-27	zip · gz · bz2
EFSA_2023	208efbd	2023-10-27	zip · gz · bz2
resfinder-4.4.0	208efbd	2023-10-27	zip · gz · bz2
resfinder-4.4.1	208efbd	2023-10-27	zip · gz · bz2
resfinder-4.4.2	208efbd	2023-10-27	zip · gz · bz2

Reporting genes and point mutations

Examples *Salmonella* – RING3S-3

69% of genes in all sequences reported by more than 90% participants

Res_Ref

Reads:

blaTEM-1B: 90.24% cov and id

blaTEM-57: 99.9% ID and 100% cov.

Assemblies:

blaTEM-1B and ***blaTEM-57***: 99.9% ID and 100% cov.

	ResFinder										AMRFinderPlus +/- ResFinder							CARD +/- others																
	Res_Ref	R05	R07	R15	R17	R23	R25	R30	R31	R33	AMR_Ref	R01	R08	R14	R35	R38	R42	R02	R10	R20	R28	R39	R40	R41	R04	R06	R21	R24	R32	% concordance
ResFinder																																	
AMRFinderPlus																																	
CARD																																	
Other																									*								
aac(6')-Iaa	X	X	X	X		X	X	X		X											X					X	X	X		41
aadA1	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		
ant(3'')-Ia	X			X	X	X				X																	X			100
aadA2	X		X	X	X	X		X	X	X	X		X	X	X		X		X	X	X	X		X	X		X	X		70
aph(3')-Ia	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	100
blaTEM-1									X					X						X					X						
blaTEM-1B	X			X																										19
blaTEM-57	X	X	X	X	X	X	X	X		X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	93
cmlA1	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	100
dfrA12	X	X	X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X	93
dfrA5	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	100
floR	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	96
fosA4	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	100
mph(A)	X		X	X	X	X	X	X	X	X	X		X	X	X		X			X	X	X		X	X	X	X	X	X	78
sul1	X	X	X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	96
sul3	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	100
tet(A)	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	96

* Bionumerics, Salmonella Resistance KB, version 2021.04.12

Coverage cut-off: 60%

aadA2: detected with 73%
Own assemblies performed

Coverage cut-off: 100%

aadA2: detected with 73%

***mphA*:** detected with 64% coverage

Examples *Salmonella* – RING3S-3

	PointFinder												AMRFinderPlus +/- PointFinder													Other		
	Res_Ref	R05	R06	R07	R15	R17	R23	R24	R25	R30	R31[A]	R33	AMR_Ref	R01	R08	R14	R35	R38	R40	R42	R02	R10	R20	R28	R32	R39	R41	R04	R21	% concordance
PointFinder																													
AMRFinderPlus																													
Other																												*	**	
gyrA D87G	X	X	X	X	X	X	X	X	X	X		X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	93
gyrA S83F	X	X	X	X	X	X	X	X	X	X		X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	93
parC T57S	X	X	X	X	X	X	X	X	X	X		X									X		X	X	X	X	X	X	X	67
parC S80I	X	X	X	X	X	X	X	X	X	X		X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	93

* Bionumerics, Salmonella Resistance KB version 2021/04.12
** RGI- 6.0.3

Only genes reported, without specifying mutations

No mutations found (same assembly as AMR_Ref)

Examples *Salmonella* – RING3S-4

	ResFinder										AMRFinderPlus +/- ResFinder																CARD +/- others				% concordance
	Res_Ref	R05	R07	R15	R17	R23	R25	R30	R31	R33	AMR_Ref	R01	R08	R14	R35	R38	R42	R02	R10	R20	R28	R39	R40	R41	R04	R06	R21	R24	R32	
ResFinder																															
AMRFinderPlus																															
CARD																															
Other																									*						
aac(3)-IIg											X	X	X	X	X	X	X		X	X	X	X	X	X	X	X			X	56
aac(6')-Iaa	X	X	X	X		X	X	X		X											X					X	X	X		41
aac(6')-Ib3	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X	96
aac(6')-IIc	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	100
aadA2	X		X	X	X	X	X[A]	X	X	X	X	X	X	X	X	X	X	X		X	X	X	X	X	X		X	X	X	89
aph(3')-Ia	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X	96
aph(3'')-Ib	X	●	X	X	X	X	X	X	X	X	X	●	X	X	X	●	X	X		X	X	X		X	X		X	X	X	78
aph(6)-Id	X	X		X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X		X	X		X	X	X	85
arr											X	X	X	X	X	X	X		X	X	X	X	X	X	X			X	X	56
blaSHV-12	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	100
blaTEM-1									X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
blaTEM-1B	X	X	X	X	X	X	X	X		X																	X			100
dfrA19	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	100
ere(A)	X	●	X	X	X	X	X	X	X	X	X	●	X	X	X	●	X			X	X	X		X			X	X	X	70
qnrB2	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	100
sul1	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	100
sul2	X	●	X	X	X	X	X	X	X	X	X	●	X	X	X	●	X		X	X	X	X		X	X		X	X	X	78
tet(B)	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X	96
tet(D)	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	100

* Bionumerics, Salmonella Resistance KB, version 2021.04.12

[A] reported as aadA2b

Coverage cut-off: 60%

Coverages for genes: between 72% and 85%

Coverage cut-off: 100%

Coverages for genes: between 72% and 85%

Examples *Campylobacter* – RING3C-1

35% of genes in all sequences reported by more than 90% participants

Reads: 94% ID and cov.

Assemblies: not detected

	ResFinder										AMRFinderPlus +/- ResFinder											CARD +/- other							
	Res_Ref	R05	R07	R15	R16	R17	R23	R25	R30	R33	AMR_Ref	R01	R04	R08	R14	R35	R38	R12	R20	R28	R39	R41	R06	R13	R21	R24	R32	R09	R37	% concordance
ResFinder																														
AMRFinderPlus																														
CARD																														
Other																								*				**	***	
blaOXA											X	●	●	○	●	X	●		X											
blaOXA-193	X	●	●	X	●	X	X	●	X	X												X		X	X					37
tet(O)								X			X	X	X	X	X	X	X	X	X	X	X		X			X			X	
tet(O/32/O)	X	X	X	X		X	X	X	X	X								X				X		X	X		X	X		
tet(O/M/O)		X	X		X														X						X		X			100

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

AMR_Ref

***blaOXA**:* possible new variant

AMR_Ref: 100% ID and 61% cov.

Cov. cut-offs: 90% or 100%

Conclusions – both EQA3 and RingTrial3

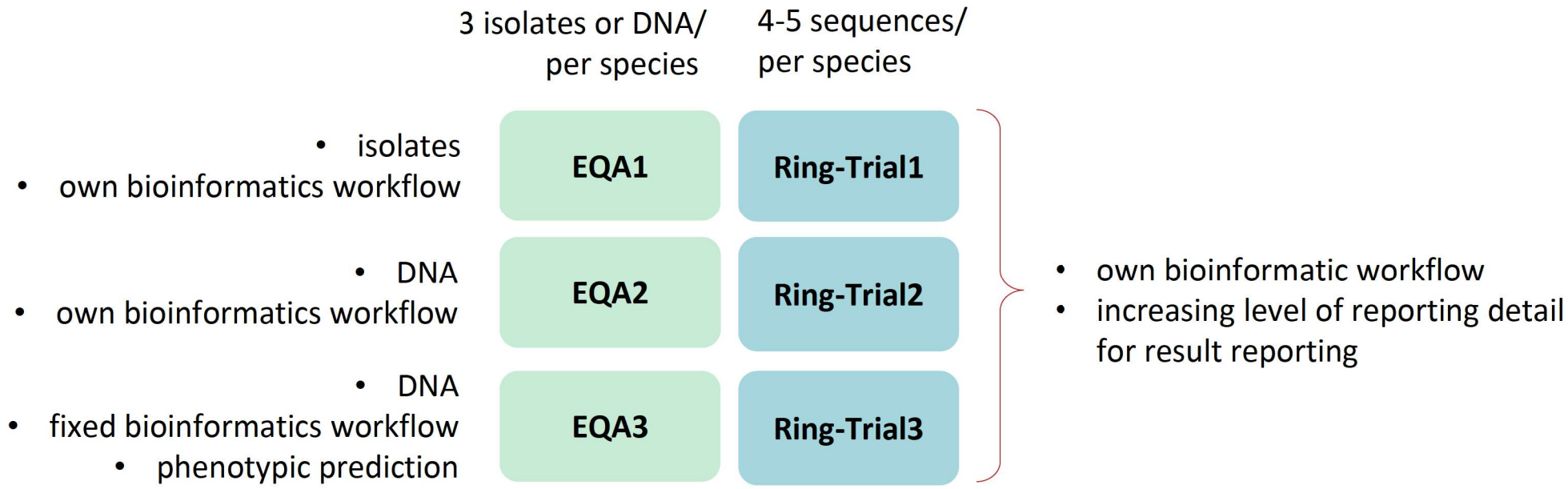
EQA:

- High quality sequences were generated by most participants
- The provided EQA3 protocol was not always followed – this resulted in a few variations
- Lower reporting rate was associated with lower sequencing quality

RT3:

- Differences in reporting due to different nomenclature or gene / PM presence in databases
- Too high cut-off values resulted in missed genes
- For *Campylobacter*: Using assemblies resulted in missed genes

Three years of EQA and RT


3 isolates or DNA/ per species
4-5 sequences/ per species
• isolates
• own bioinformatics workflow
EQA1
Ring-Trial1
• DNA
• own bioinformatics workflow
EQA2
Ring-Trial2
• DNA
• fixed bioinformatics workflow
• phenotypic prediction
EQA3
Ring-Trial3
• own bioinformatic workflow
• increasing level of reporting detail for result reporting

Lessons learned

Tools vs databases (input restrictions, version reporting)

Different nomenclature in different databases

Reporting strategies

Different results depending on input files (fastq vs fasta)

Cut-offs

Sequencing quality

Following the protocol (if given)

Human errors

How to improve future EQAs

Well-defined thresholds for sequencing quality

Individual feedback – dialogue with participants to validate results

More defined bioinformatics parameters (e.g. cut-offs) depending on the purpose of the exercise

Better quality of reference datasets used in the exercises (closed genomes)

Other ideas?

Thank you for your attention

Colleagues from SSI

Eva Møller Nielsen
Mia Torpdahl
Susanne Schjørring
Jeppe Boel
Anne Sophie Majgaard Uldall
Karen Loaiza Conza

Colleagues from DTU

René Hendriksen
Susanne Karlsmose Pedersen
Ana Rita Bastos Rebelo
Elif Seyda Tosun
Sarah Marvig Johansson