
DTU

Carbacamp

Assessment of phenotypic carbapenem susceptibility and genomic epidemiology of *Campylobacter* from animal, food and human domains

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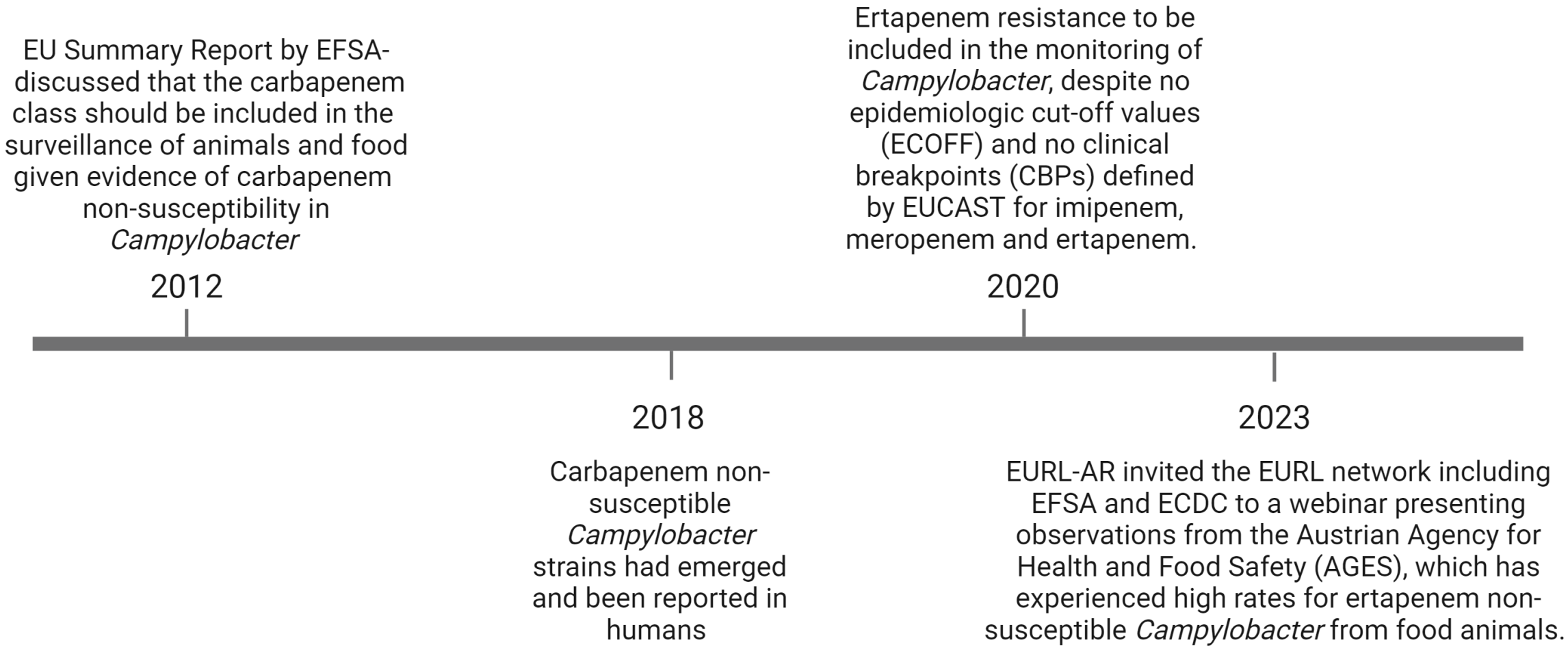
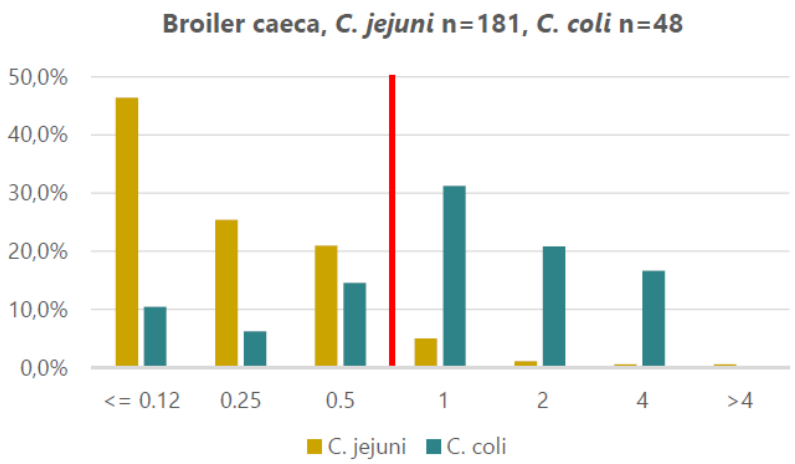
Carbacamp: Background

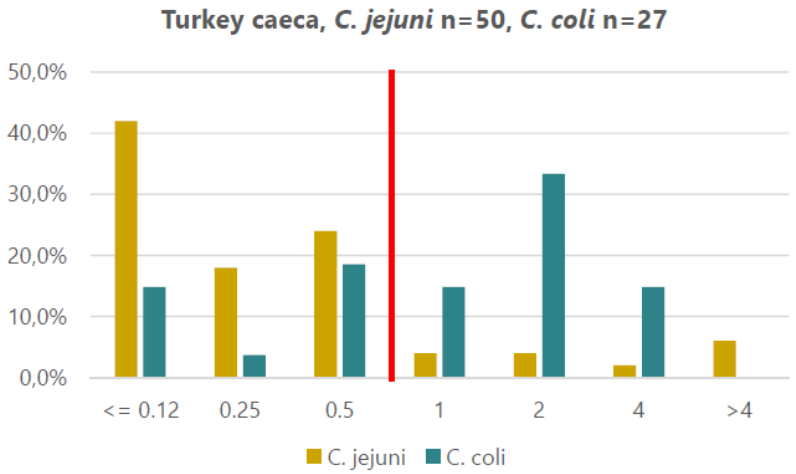


Carbacamp: Background

Resistance rates for ertapenem



Threshold	*C. jejuni*	*C. coli*
R>0.5	7.2 %	**68.8 %**

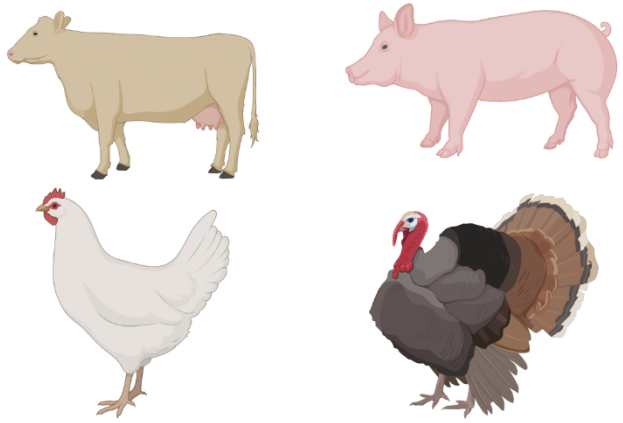


Threshold	*C. jejuni*	*C. coli*
R>0.5	16.0 %	**63.0 %**

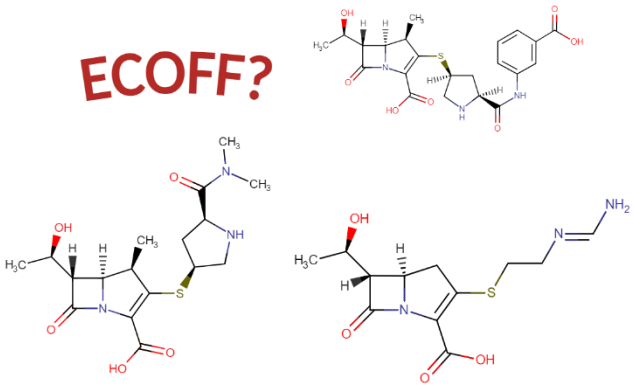
- The monitoring data showed possible different wild-type distributions between *C. jejuni* and *C. coli,* as well as in between different animal types of the same species.

Carbacamp: Objectives

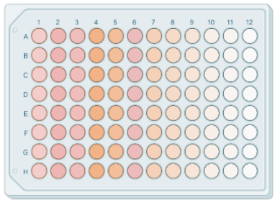
Determine WT distribution between *C. jejuni* and *C. coli*, as well as 4 animal domains, and human isolates



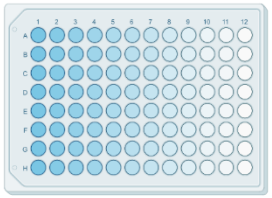
Set epidemiological cut-off values (ECOFF) for imipenem, ertapenem and meropenem for DD and MIC



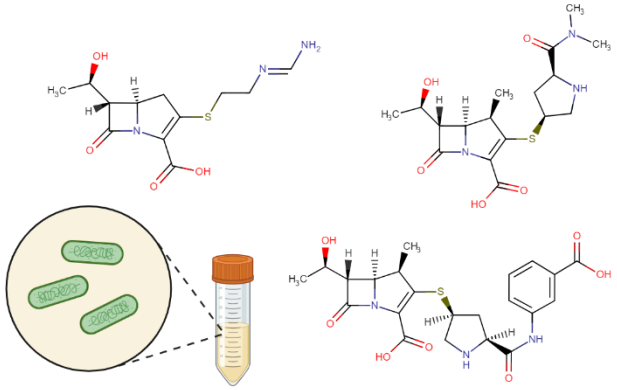
Determine comparability of EUCAST and CLSI recommended media for *Campylobacter* MIC



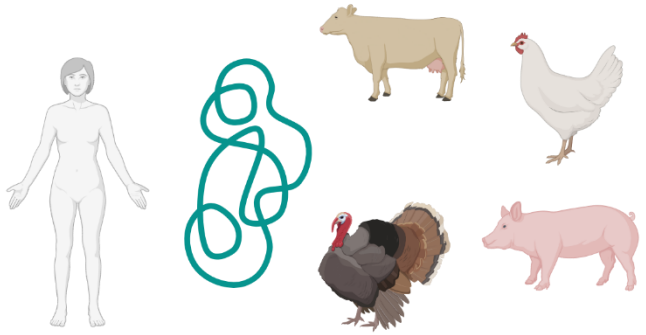
EUCAST (MH-F)



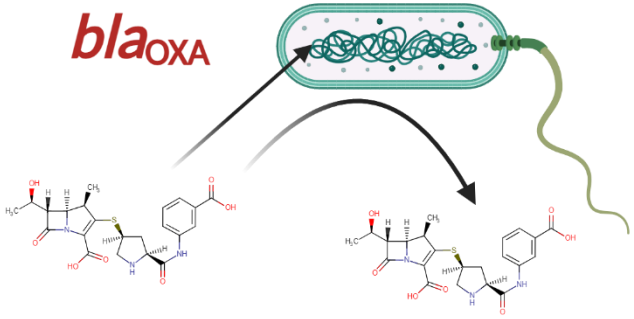
CLSI (MH+LHB)



Determine which of ertapenem, imipenem and meropenem are most suitable for the monitoring of *Campylobacter.*



Determine the genomic diversity (clustering) of susceptible and non-susceptible *C. jejuni* and *C. coli* across the animal species and humans



Investigate potential resistance mechanism conferring non-susceptibility to carbapenems as well as the role of bla_{OXA} genes

CarbaCamp: Tasks

Task 1	**Collection of strains** -2.400 *Campylobacter* isolates will be collected from at least six countries -data availability are a key principle -each country will provide 55 isolates of each species *C. jejuni* and *C. coli* from four animal and food domains 1) broilers meat and caeca 2) turkey caeca 3) pig and pork 4) cattle and beef
Task 2	**Antimicrobial susceptibility testing by disk diffusion** -200 of the 2.400 isolates will be shipped to the EUCAST EDL for initial disk diffusion testing by EDL and DTU employees to ensure high quality proficiency reading and analysis -remaining 2.200 isolates will be tested at DTU using Mueller Hinton agar from two suppliers: Oxoid and BioRad (BD)
Task 3	**Antimicrobial susceptibility testing by MIC determination** -2.400 isolates will be reduced to subset of 600 isolates based on DD results suggested by EDL to be MIC determined with custom sensititre plate design at DTU
Task 4	**Sequencing** -the subset of 600 isolates tested by MIC will be whole genome sequenced using Illumina
Task 5	**Bioinformatic analysis** -raw data of study will be supplemented with C. jejuni and C. coli genomes from ENA -genomes will be analysed for presence of known antimicrobial determinants and investigated for novel resistance mechanisms
Task 6	**Sharing of data** -data will be shared for all participants, with eventual release in public domain

Annex 1: CarbaCamp Gantt chart

		Months
		Responsible
1	Kick off meeting	DTU
2	Develop survey, recruit laboratories to ship the strains to DTU	DTU
3	Subculture strains at the NRL's and prepare for shipment to the EURL	DTU/NRL
4	Signature of MTA's, collect strains in a database and blind strain codes	DTU
5	Receive strains at DTU, ensure purity and store	DTU
6	Shipment of 200 strains to EDL	DTU
7	Disk diffusion at EDL (first 200 isolates)	EDL/DTU
8	Disk diffusion at DTU (remaining 2200 isolates)	DTU
9	Select final 600 strains	EDL/DTU
10	Perform broth microdilution (1000 strains)	DTU
11	DNA extraction and sequencing (600 isolates)	DTU
12	QC and bioinformatics analysis	DTU
13	Comparison of data (DD, MIC, WGS)	DTU
14	Interim meetings	DTU
15	Interim report	DTU
16	Final report	DTU

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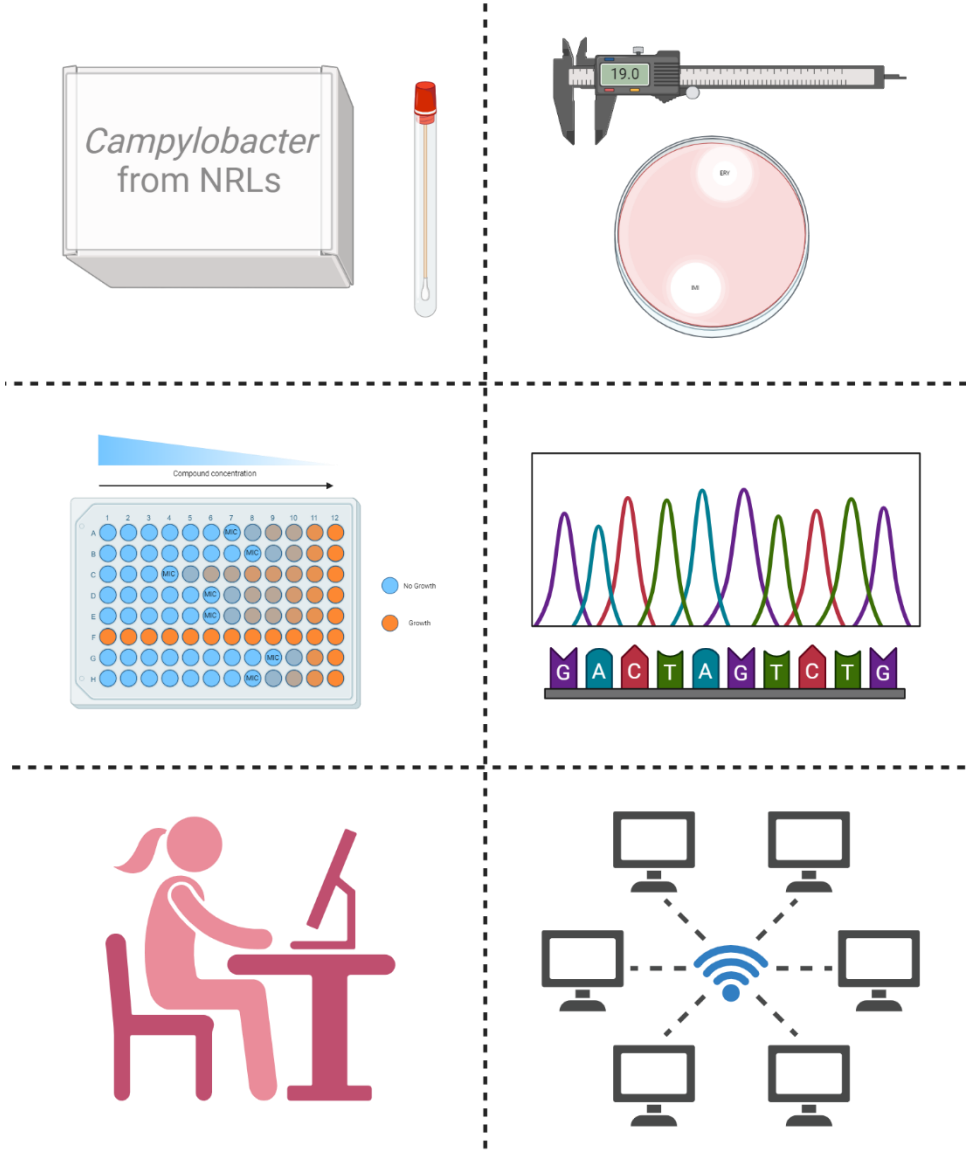
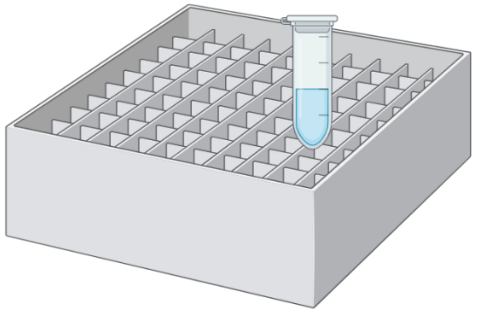
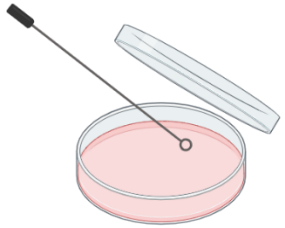


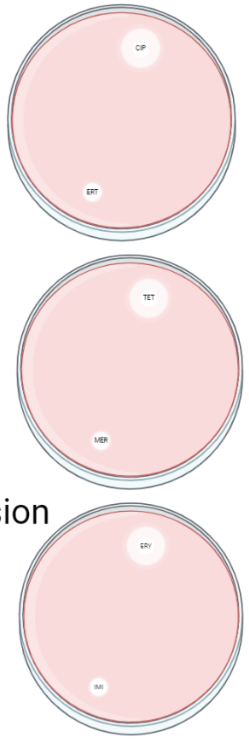
Disk Diffusion CarbaCamp



Day 1: revive strains from -80C

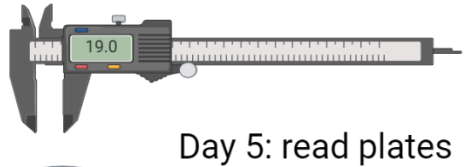


Day 3: Subculture



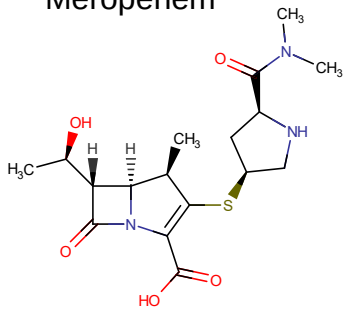
Day 4: disk Diffusion

3 plates/isolate

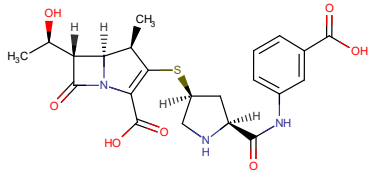


Day 5: read plates

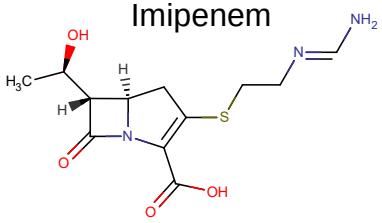
Meropenem



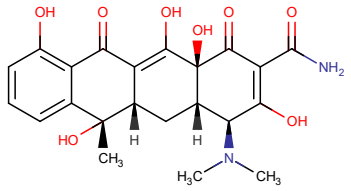
Ertapenem



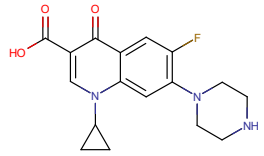
Imipenem



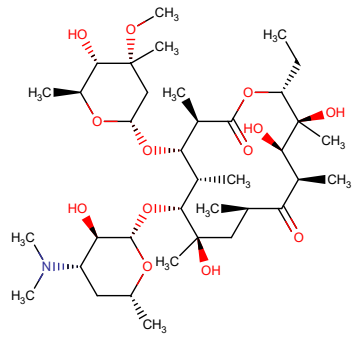
Tetracycline



Ciprofloxacin



Erythromycin



MIC Custom Panels

SENSITITRE CUSTOM PLATE FORMAT

1905201122

Plate Code: **DNK1CAMP** Date: **12-Mar-24**

	1	2	3	4	5	6	7	8	9	10	11	12
A	ETP 0.002	ETP 0.5	IMI 0.002	IMI 0.5	MERO 0.002	MERO 0.5	ETP 0.002	ETP 0.5	IMI 0.002	IMI 0.5	MERO 0.002	MERO 0.5
B	ETP 0.004	ETP 1	IMI 0.004	IMI 1	MERO 0.004	MERO 1	ETP 0.004	ETP 1	IMI 0.004	IMI 1	MERO 0.004	MERO 1
C	ETP 0.008	ETP 2	IMI 0.008	IMI 2	MERO 0.008	MERO 2	ETP 0.008	ETP 2	IMI 0.008	IMI 2	MERO 0.008	MERO 2
D	ETP 0.015	ETP 4	IMI 0.015	IMI 4	MERO 0.015	MERO 4	ETP 0.015	ETP 4	IMI 0.015	IMI 4	MERO 0.015	MERO 4
E	ETP 0.03	ETP 8	IMI 0.03	IMI 8	MERO 0.03	MERO 8	ETP 0.03	ETP 8	IMI 0.03	IMI 8	MERO 0.03	MERO 8
F	ETP 0.06	ETP 16	IMI 0.06	IMI 16	MERO 0.06	MERO 16	ETP 0.06	ETP 16	IMI 0.06	IMI 16	MERO 0.06	MERO 16
G	ETP 0.12	ETP 32	IMI 0.12	IMI 32	MERO 0.12	MERO 32	ETP 0.12	ETP 32	IMI 0.12	IMI 32	MERO 0.12	MERO 32
H	ETP 0.25	POS	IMI 0.25	POS	MERO 0.25	POS	ETP 0.25	POS	IMI 0.25	POS	MERO 0.25	POS

ANTIMICROBICS

ETP	Ertapenem
IMI	Imipenem
MERO	Meropenem
POS	Positive Control

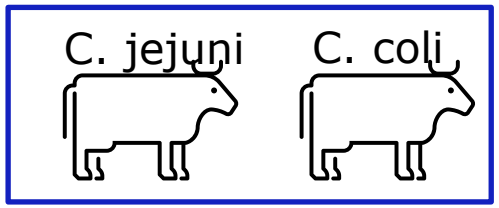
- 600 isolates with MH-F (EUCAST recommendation)
- 400 isolates with MH+LHB (CLSI recommendation)
- 100 human isolates

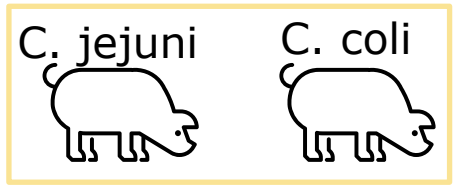
Isolate selection: summary

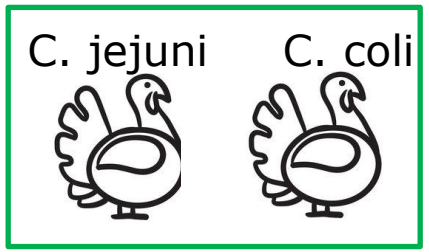
- There are two isolate sub-collections:
  - Isolates selected for **ECOFF determination**, 20 (15+5) from WT population and 35 from non-WT.

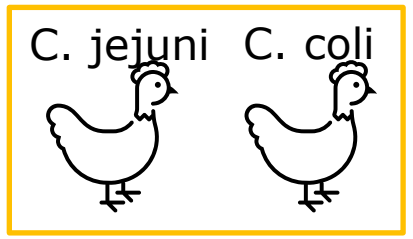
Strategy	Selection of "WT" isolates (MIC_{ETP}<=0.125)		Selection of "non-WT" isolates (MIC_{ETP}>0.125)		Total nr of isolates
	Selection principle	Nr of isolates	Selection principle	Nr of isolates	
1	Random selection.	20 (15+5 for safety) or all available, if between 15-19 isolates for some cases.	Proportionate selection from each MIC value. See example below*.	30-35	55

  - Isolates selected for **genetic analyses of AMR mechanism**, for MIC to ETP equal or greater than 2 mg/L.
- We target **8 Campylobacter species/host combinations**, each with a **max target of 300 isolates => 2400 TOTAL nr of isolates.**

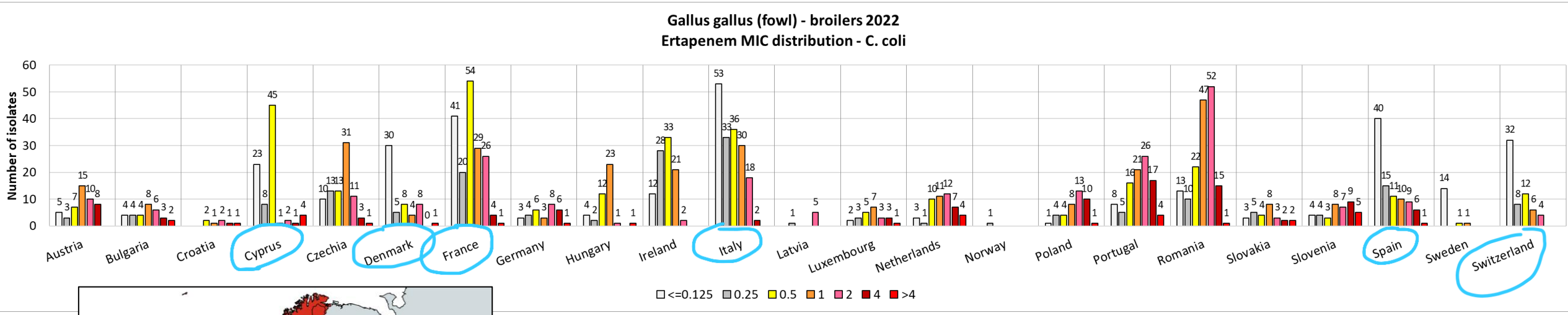








Isolate selection example: Broilers *C. coli*



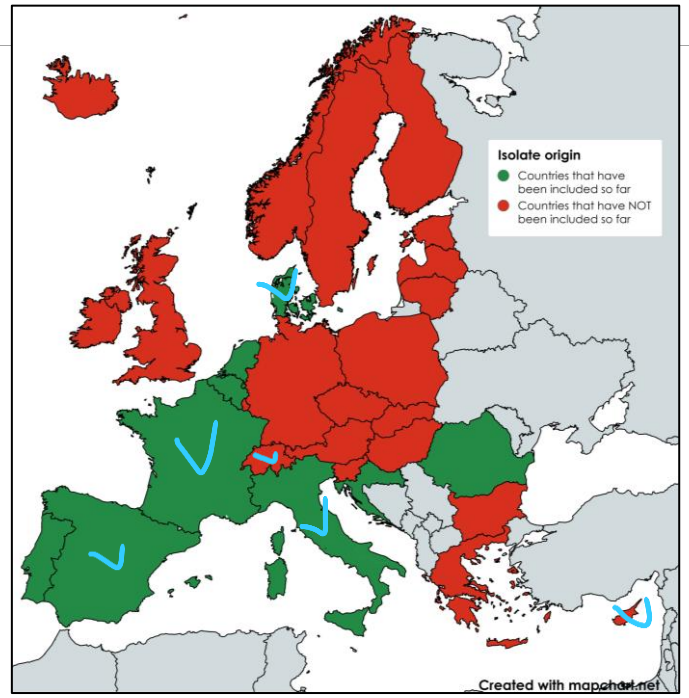
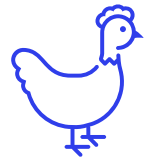
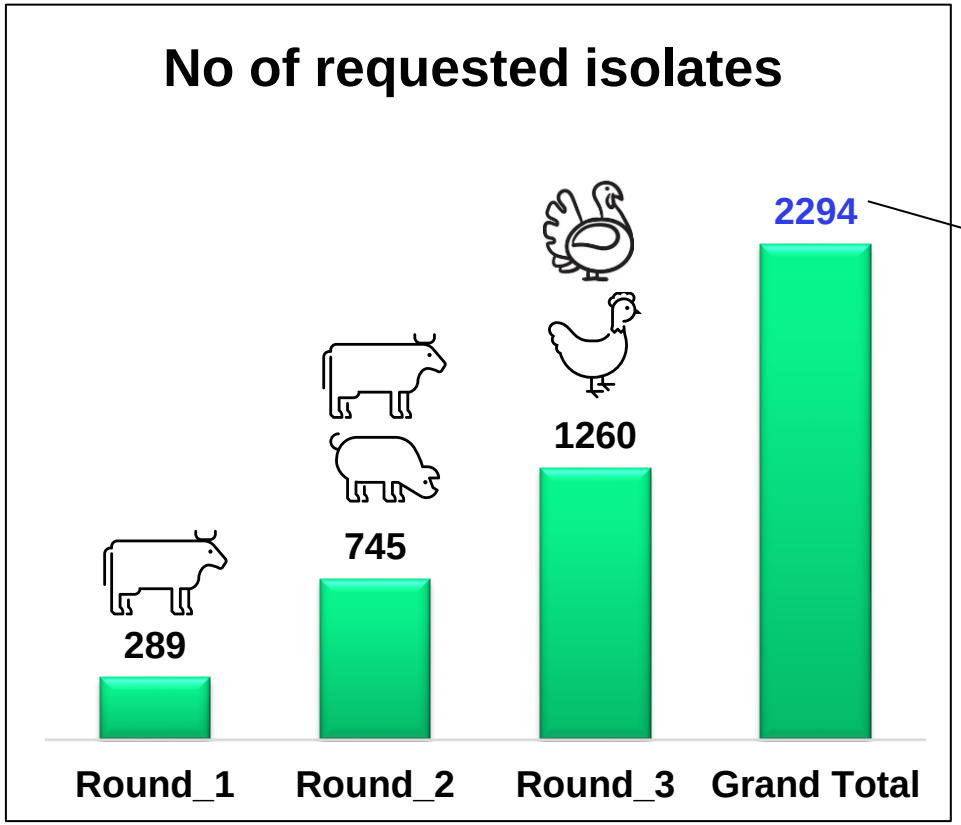


Figure: Bar graph of ertapenem MIC values of *C. coli*, isolated from broilers from 2022 EU AMR monitoring data.

Map: Geographical spread of selected isolates for *C. coli*/broiler host/species combination



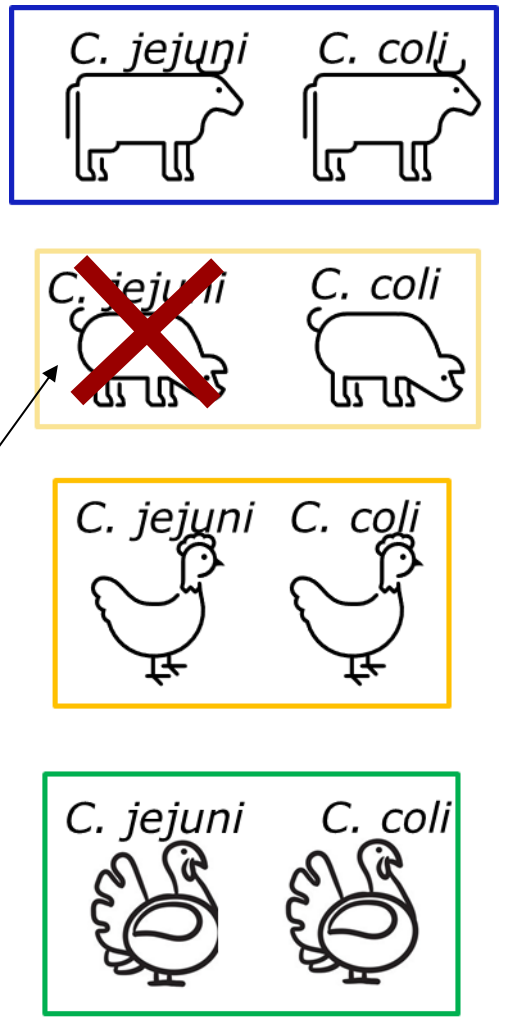
Three collection rounds for requesting 96% of target no of isolates



Target n=300 isolates per host/species => **2400** total

96% of target number requested

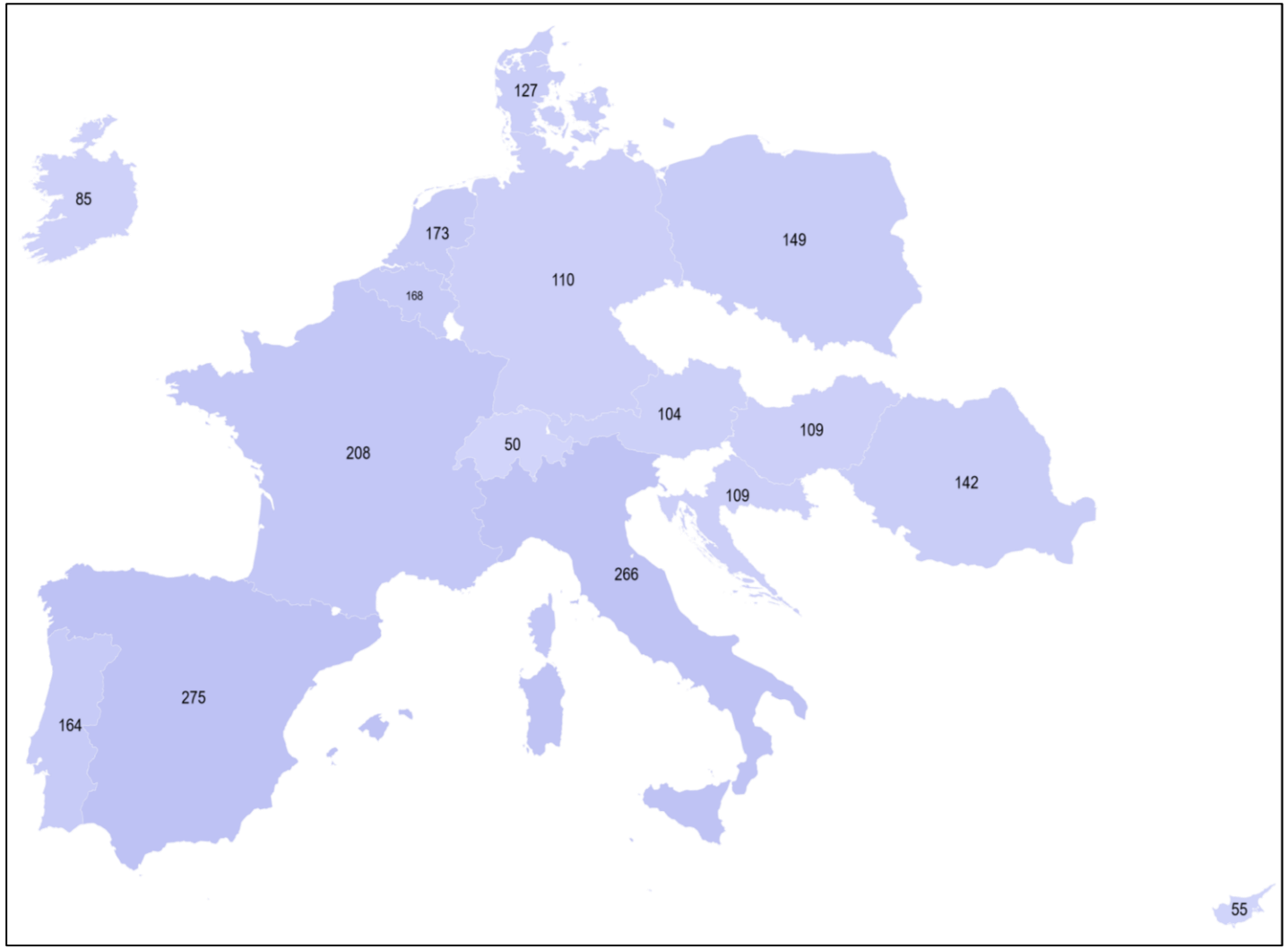
Host	*C. coli*	*C. jejuni*
Cattle	289	320
Broilers	316	330
Pigs	425	no isolates
Turkeys	313	301



CarbaCamp isolates originate from 16 countries

At least 6 countries

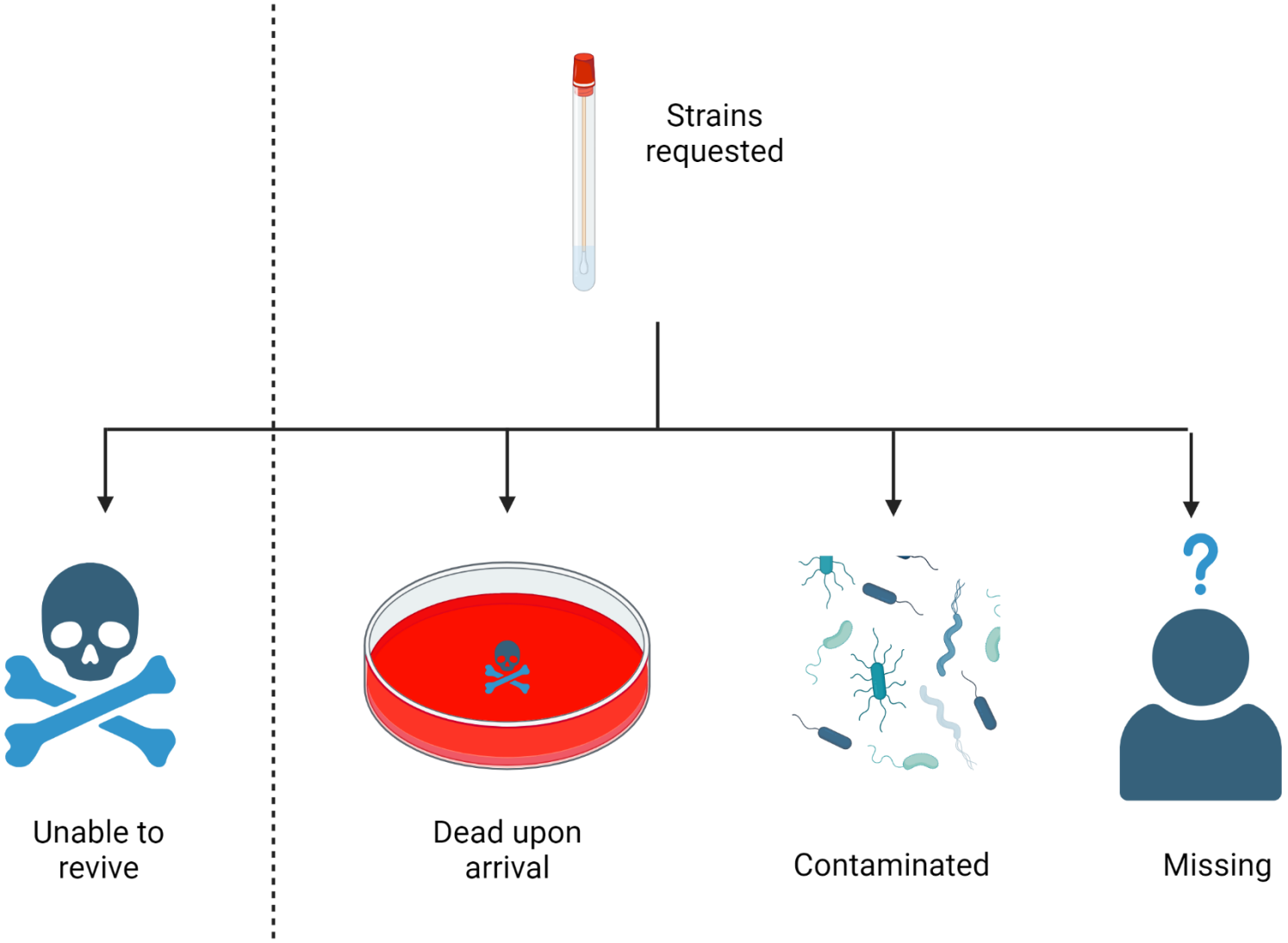
Country (n=16)	No of isolates (High to low)
Spain	275
Italy	266
France	208
Netherlands	173
Belgium	168
Portugal	164
Poland	149
Romania	142
Denmark	127
Germany	110
Croatia	109
Hungary	109
Austria	104
Ireland	85
Cyprus	55
Switzerland	50
Grand Total	**2294**



Isolate Collection: Loss of isolates overview

Prior to arriving at DTU

After arriving at DTU



Requested versus QC passed isolates

	No of requested isolates		No of isolates Passed QC		No of "lost" isolates		% "Lost" isolates	
Host	***C. coli***	***C. jejuni***	***C. coli***	***C. jejuni***	***C. coli***	***C. jejuni***	***C. coli***	***C. jejuni***
Cattle	289	320	206	282	83	38	29	12
Broilers	316	330	159	322	157	8	50	2
Pigs	425	-	350	-	75	-	18	-
Turkeys	313	301	197	278	116	23	37	8
Total	**1343**	**951**	**912**	**882**	431	69	**32**	**7**

2294 total

1794 total

500 isolates (22% of requested) "lost"

- **Dead at NRL**
- **Not received**
- **Fail internal QC**

11% of requested isolates not received mainly due to being dead at NRL

Isolate received?	Reason	No of isolates	% of all isolates
No	**Total**	**250**	**10.90**
	Dead at NRL	196	8.54
	Collection cancelled - too few live isolates in this host/species combination	42	1.83
	Poor growth at NRL	8	0.35
	Not in the parcel	2	0.09
	Contamination at NRL	1	0.04
	Did not pass QC at NRL	1	0.04
Yes	-	2044	89.10
	Total	**2294**	

196 out of 250 (78%) of not received isolates were dead at NRL

Replacement of isolates reported dead at NRL

- Some NRLs informed us before the shipment about the dead isolates:

  => n=26 could be replaced, new isolates requested

  => n=45 **could not** be replaced

- Some NRLs did not inform us in advance so we need to check if the dead isolates can be replaced (n=194)

- **New total no of strains requested = 2320** (2294 initially + 26 dead substitutes)

- **5 of the 26 substitutes were also dead at the NRL ☺**

Isolate received?	Number
No	255
Yes	2065
Total	**2320**

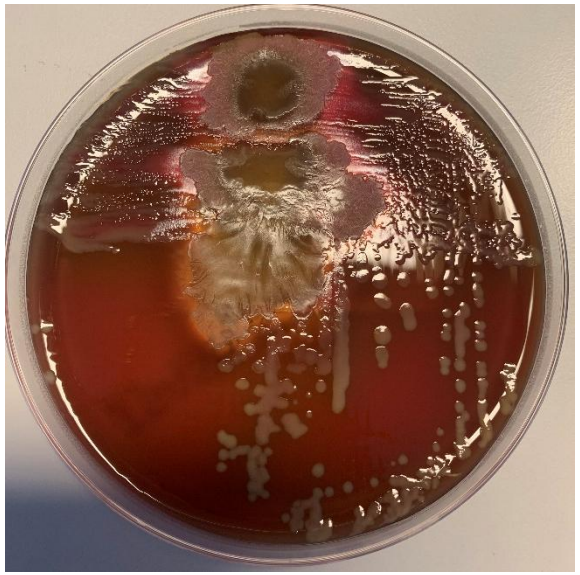
Internal QC of isolates (purity, Campylobacter species)

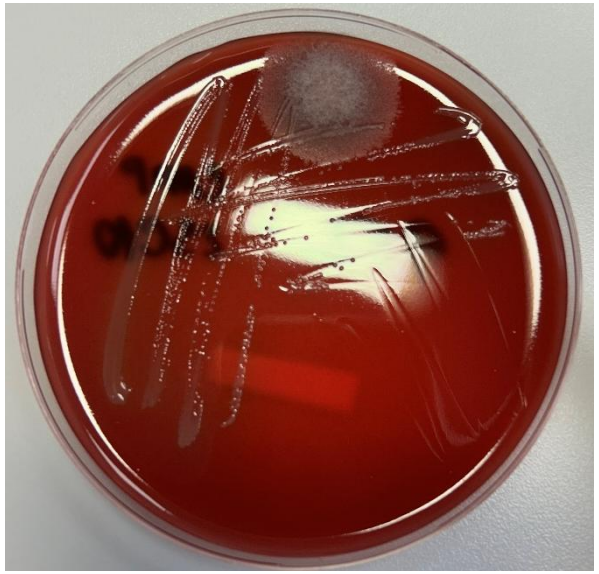
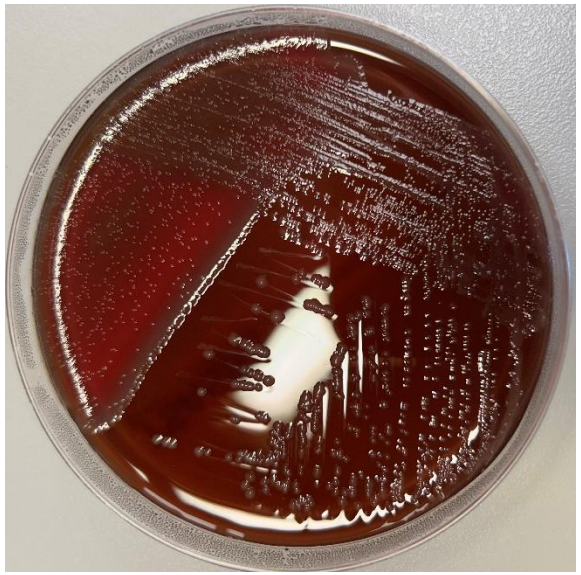
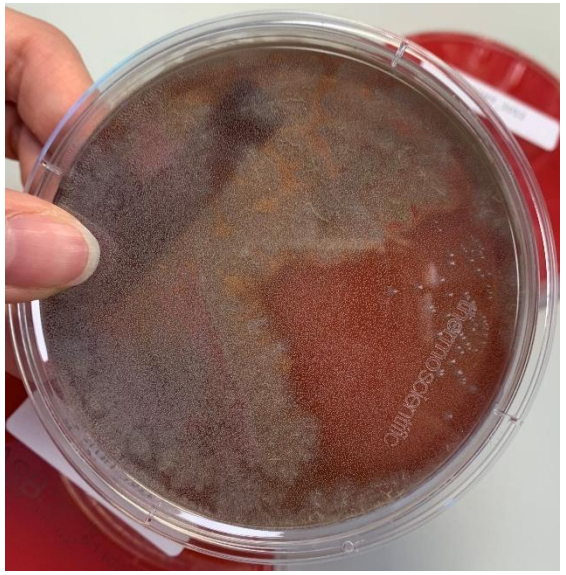
Alive upon arrival?	yes

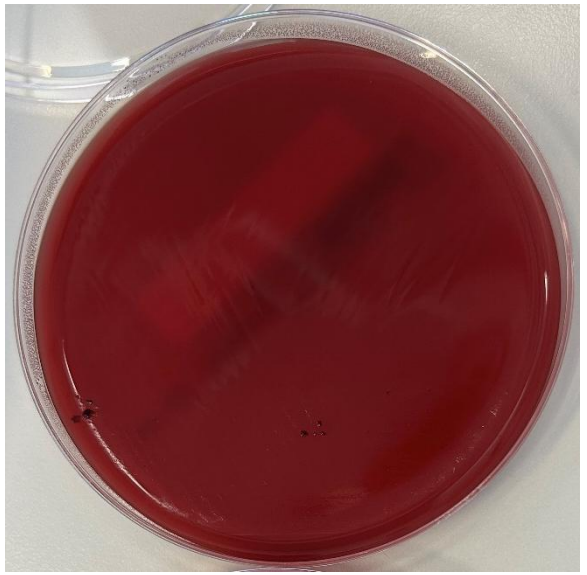
Internal QC Results	No of isolates	%	
Passed QC	**1794**	**95.4**	591 run in DD (33%)
Contaminated	**41**	**2.2**	
Internal QC pending	21	1.1	
Unexpected Campylobacter species, CC => CJ	16	0.9	Can't use them
Unexpected Campylobacter species, CJ => CC	5	0.3	
Species changed from the country from CJ to CC	3	0.2	
Total	**1880**		

Contamination type	No
Not defined	31
Lactobacillus	2
Micrococcus luteus	1
Staphylococcus epidermidis	1
Staphylococcus haemolyticus	1
Staphylococcus hominis	1
Micrococcus lylae	1
Ureibacillus acetophenoni	1
Roseomonas mucosa	1
Staphyloccoccus epidermidis	1

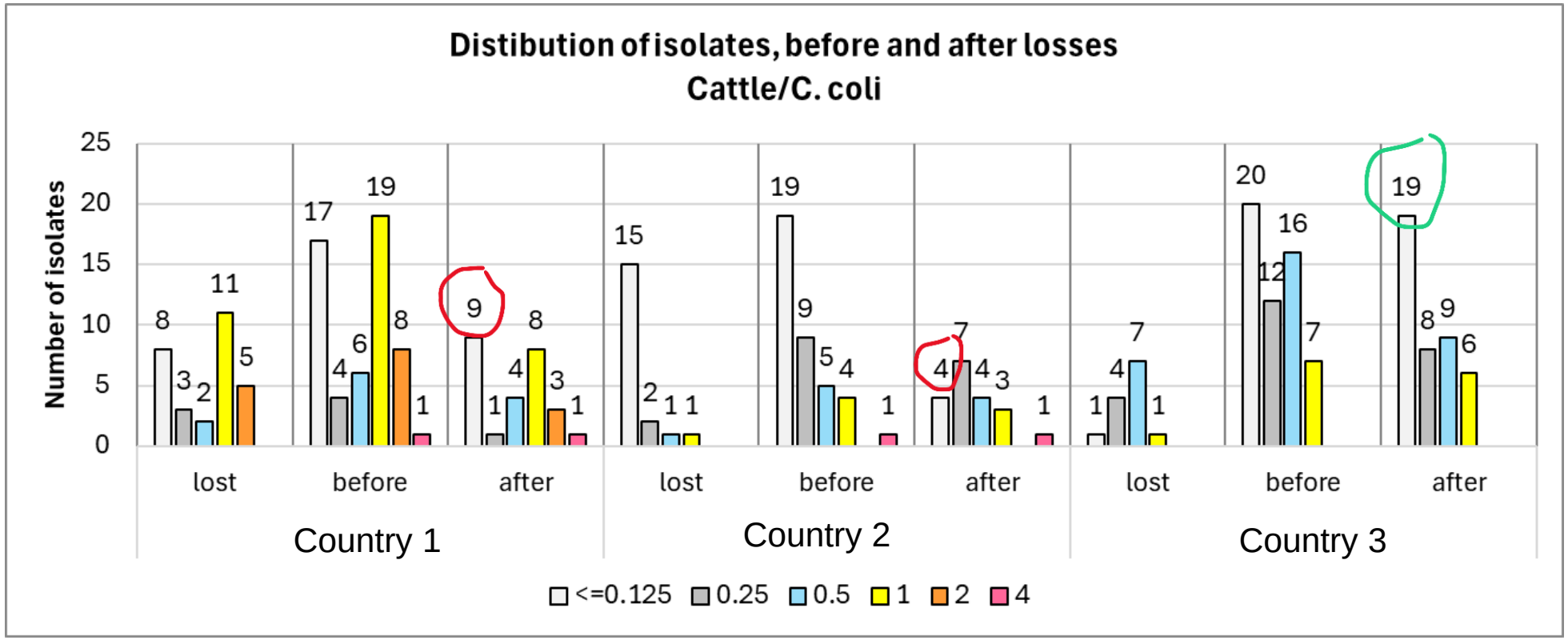
Isolate Collection Examples



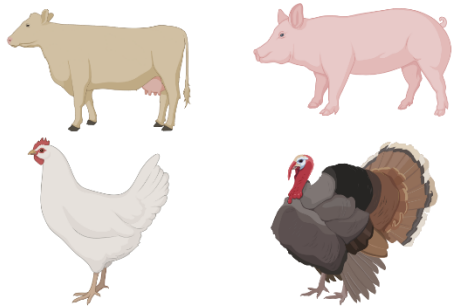


Lost isolates per MIC to ETP – example Cattle/C. coli

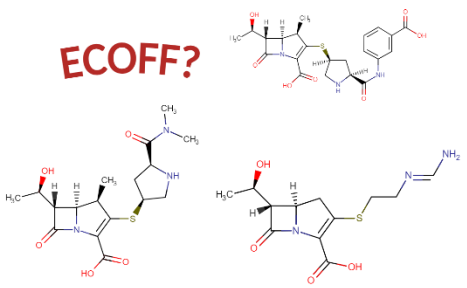


Summary

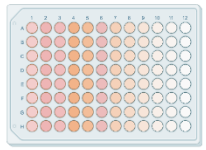
Determine WT distribution between *C. jejuni* and *C. coli*, as well as 4 animal domains, and human isolates



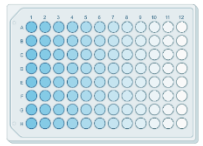
Set epidemiological cut-off values (ECOFF) for imipenem, ertapenem and meropenem for DD and MIC



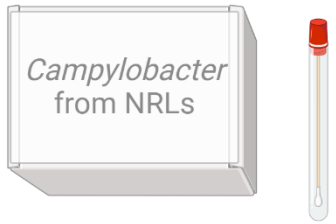
Determine comparability of EUCAST and CLSI recommended media for *Campylobacter* MIC

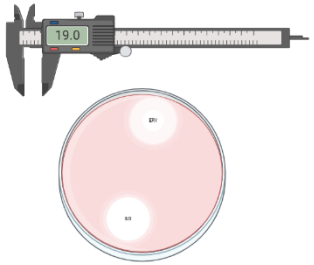


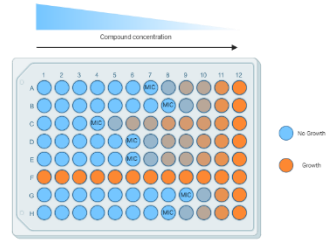
EUCAST (MH-F)

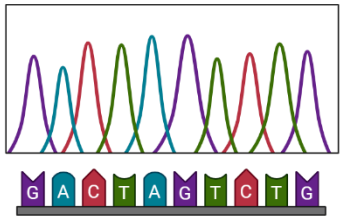


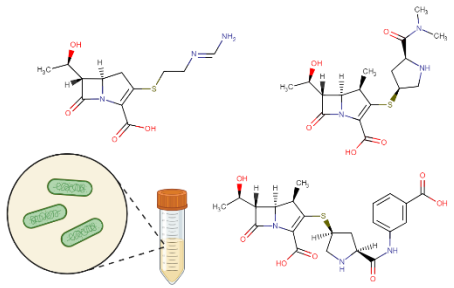
CLSI (MH+LHB)



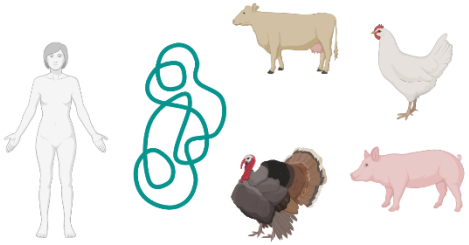




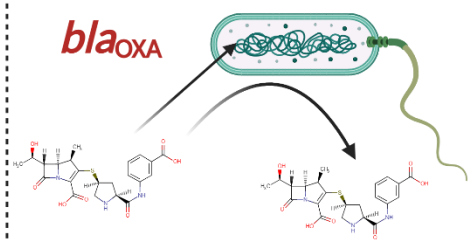




Determine which of ertapenem, imipenem and meropenem are most suitable for the monitoring of *Campylobacter.*



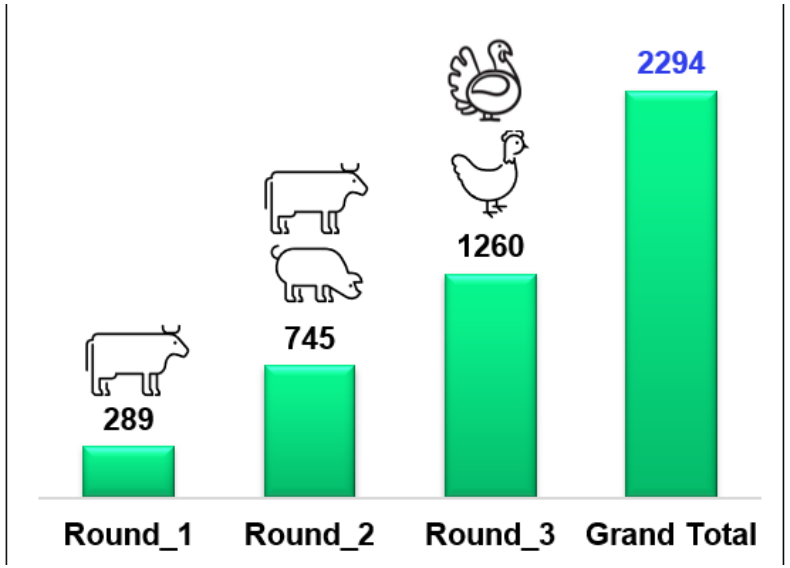
Determine the genomic diversity (clustering) of susceptible and non-susceptible *C. jejuni* and *C. coli* across the animal species and humans



Investigate potential resistance mechanism conferring non-susceptibility to carbapenems as well as the role of bla_{OXA} genes

Summary

Isolate collection



- 96% of target number of isolates requested
- Isolates originate from 16 EU countries
- 591 isolates have had DD run

Acknowledgements

- **DTU**
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- Raquel Garcia Fierro

EUCAST EUROPEAN COMMITTEE ON ANTIMICROBIAL SUSCEPTIBILITY TESTING
European Society of Clinical Microbiology and Infectious Diseases

efsa EUROPEAN FOOD SAFETY AUTHORITY

DTU