Genomic epidemiology study of MDR *Campylobacter coli* isolated in the context of One Health

Mónica Oleastro

National Reference Laboratory for Gastrointestinal Infections

National Institute of Health, Portugal

REPÚBLICA PORTUGUESA
SAÚDE

SNS SERVIÇO NACIONAL DE SAÚDE

Instituto_Nacional de Saúde
Doutor Ricardo Jorge

Human campylobacteriosis, PT:

Campylobacteriosis has been the most frequently reported zoonosis in humans across the EU since 2005.

In PT: A total of **5200 *Campylobacter* spp. isolates studied in the NRL**, **between 2009-2021**, from North, Center and Lisbon/Tagus Valley.

C. jejuni vs C. coli:

C. jejuni accounted for 87.4% - 89.2%

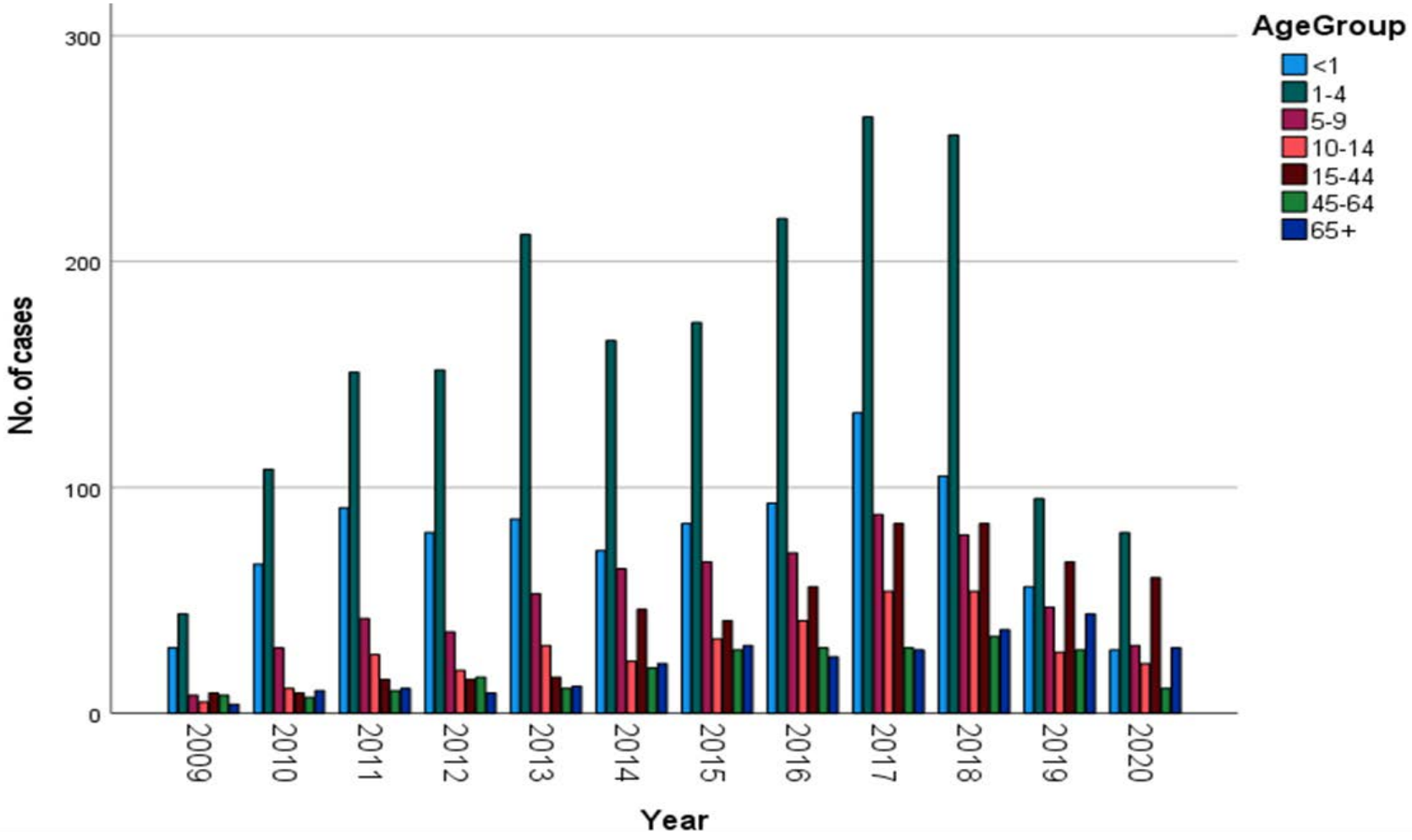
C. coli, representing 10.8% - 12.6%($P < 0.001$)

Patients' mean age was 12 years old (range: 1 day-96 years old)

Male vs Female: **Association between campylobacteriosis and gender was statistically significant ($p<0.001$)**, male (60.5%) vs female (39.5%)

Human campylobacteriosis, PT:

Distribution of campylobacteriosis by age group, 2009-2020



⇨ ***C. coli* infections are more frequent in adults (18.1%) than in children (9.9%).**

Human campylobacteriosis, PT:

	No. positive samples by species (% of total) by clinical sample	
Clinical sample used for identification of *Campylobacter*	*Campylobacter jejuni*	*Campylobacter coli*
Stool	4274 (87.6)	561 (11.5)
Blood	37 (0.8)	6 (0.1)
Gastric biopsy	1 (0.02)	0 (0)
Bile	0 (0)	1 (0.02)

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Main symptoms	**Paediatric population**	**Adult population**	**Total**	**OR (95%CI)**	**p-value**
	n (% within group)	n (% within group)			
Bloody Diarrhoea	1311 (45.4)	92 (15.1)	1403	0.213 (0.169-0.269)	**<0.001**
Diarrhoea	1442 (50.0)	400 (65.5)	1842	1.897 (1.581-2.276)	**<0.001**
Abdominal pain	332 (11.5)	120 (19.6)	452	1.877 (1.491-2.362)	**<0.001**
Fever	376 (13.0)	106 (17.3)	482	1.402 (1.107-1.774)	**0.005**
Vomiting	171 (5.9)	45 (7.3)	216	1.263 (0.898-1.775)	0.179

Human campylobacteriosis, PT, antimicrobial resistance:

Antimicrobial category	Antimicrobial agent	% Resistance (n) in *Campylobacter*			p-value
		Total (N=1849)	*C. jejuni* (N=1529)	*C. coli* (N=320)	
β-lactams	Ampicillin	74.9	74.3	78.6	0.278
Fluoroquinolones	Ciprofloxacin	94.4	94.0	95.9	0.182
Macrolides	Erythromycin	**11.8**	**3.3**	**52.2**	<0.001
Aminoglycosides	Gentamicin	**0.5**	**0.1**	**2.5**	<0.001
Tetracyclines	Tetracycline	**81.4**	**79.2**	**92.2**	<0.001

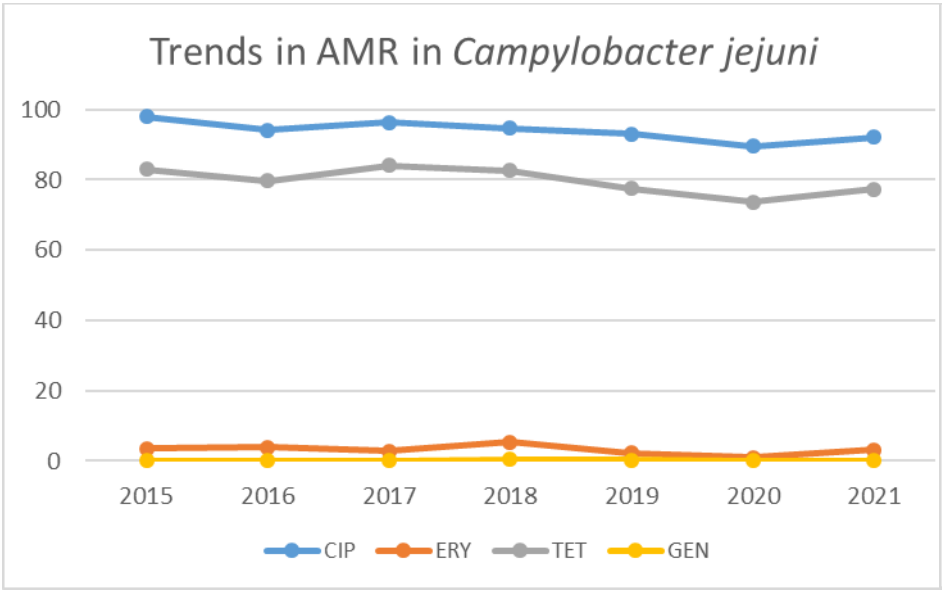
AST method: disk diffusion (EUCAST 2022)

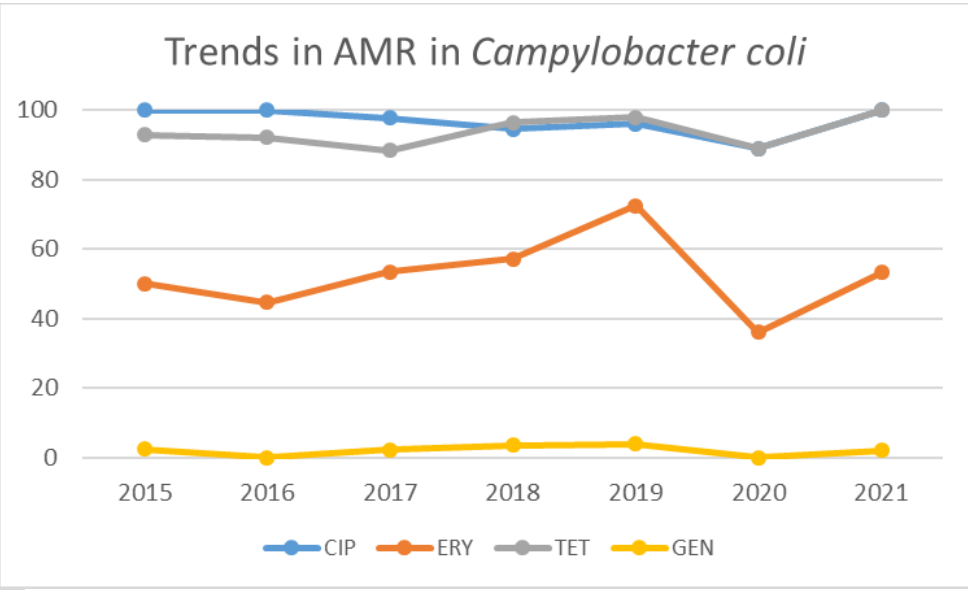
Associated resistant determinants (n=250) were:

23S rRNA (A2075G & 2074A>T*****), **no *ermB (ribosomal RNA methyltransferase)***
gyrA (T86I) (T86I, C86I), (T86I, D90N)
tetO

*****Rare mutation

Human campylobacteriosis, PT, antimicrobial resistance:



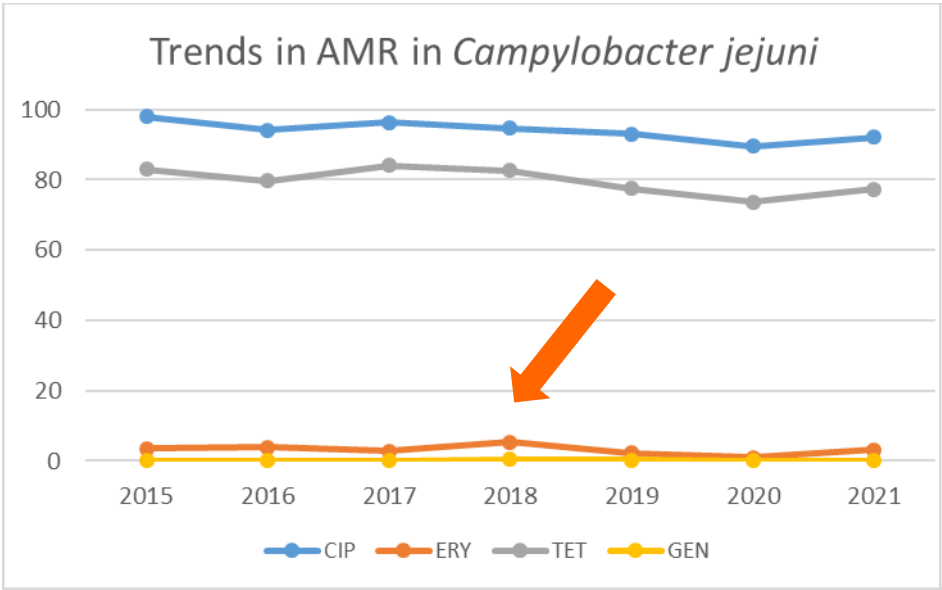


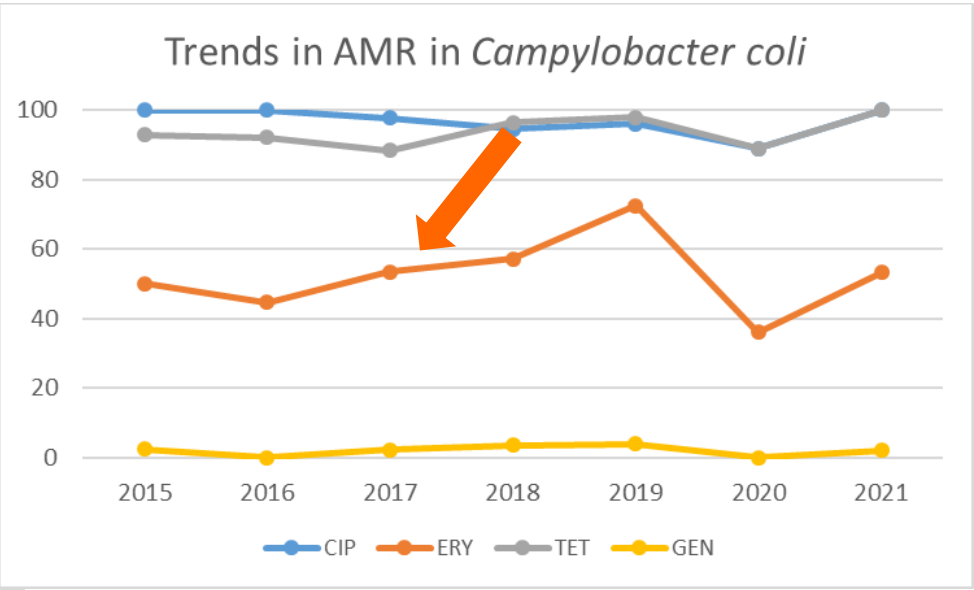
N. of isolates

	2015	2016	2017	2018	2019	2020	2021
C. jejuni	140	153	246	280	298	212	278
C. coli	42	38	43	56	51	36	47

Extremely high levels of resistance to CIP and TET, for both *C. jejuni* and *C. coli*, with a stable trend.

Human campylobacteriosis, PT, antimicrobial resistance:



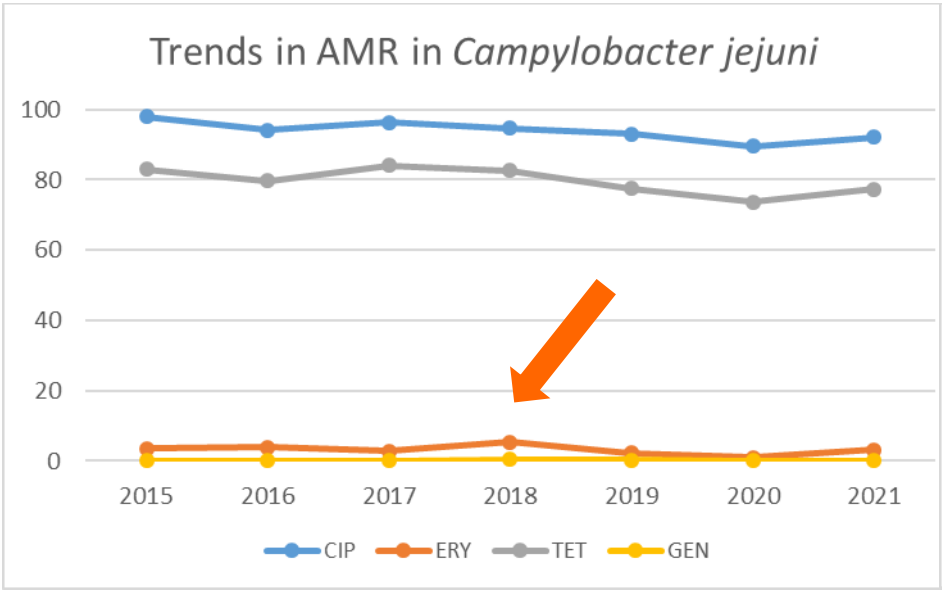


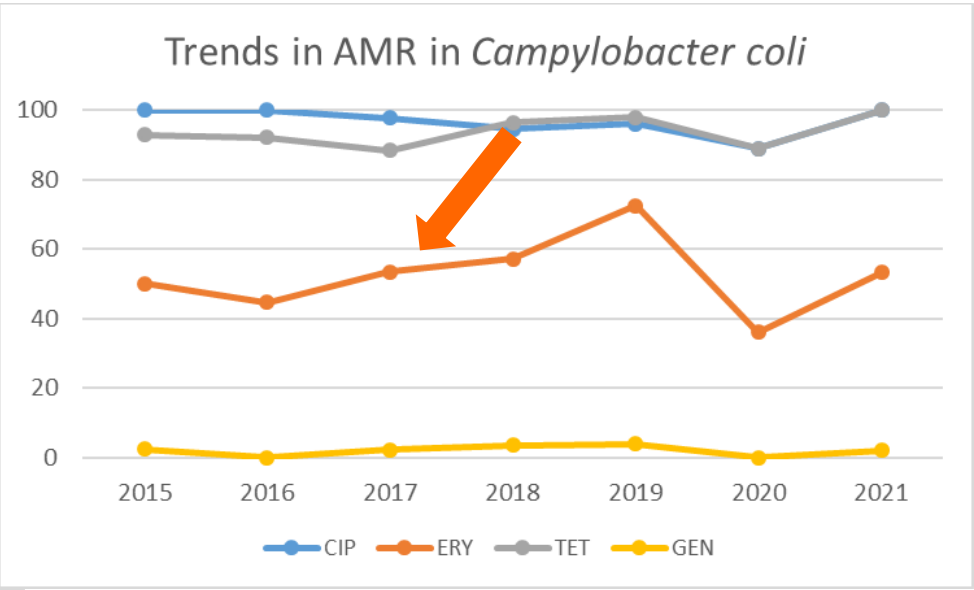
Resistance to erythromycin:

For both species, no inhibition zone was observed around ERY disc **(=6 mm inhibition zone**), which corresponds to a MIC of ≥ 128 mg/L in most cases. **HIGH-LEVEL RESISTANCE**

1- EFSA and ECDC 2021. The European Union Summary Report on Antimicrobial Resistance in zoonotic and indicator bacteria from humans, animals and food in 2018/2019. EFSA Journal 2021;19(4):6490, 179 pp. https://doi.org/10.2903/j.efsa.2021.6490

Human campylobacteriosis, PT, antimicrobial resistance:



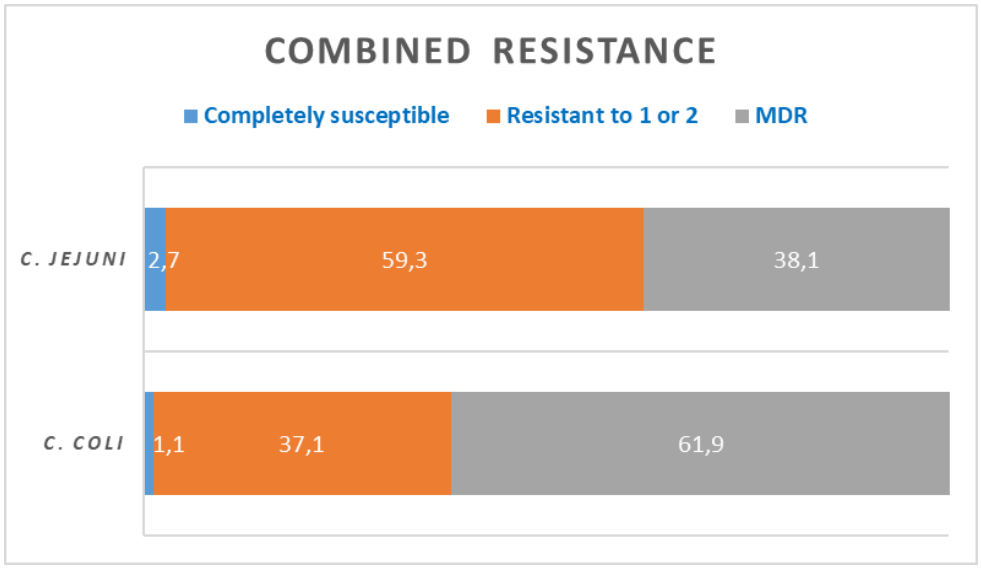


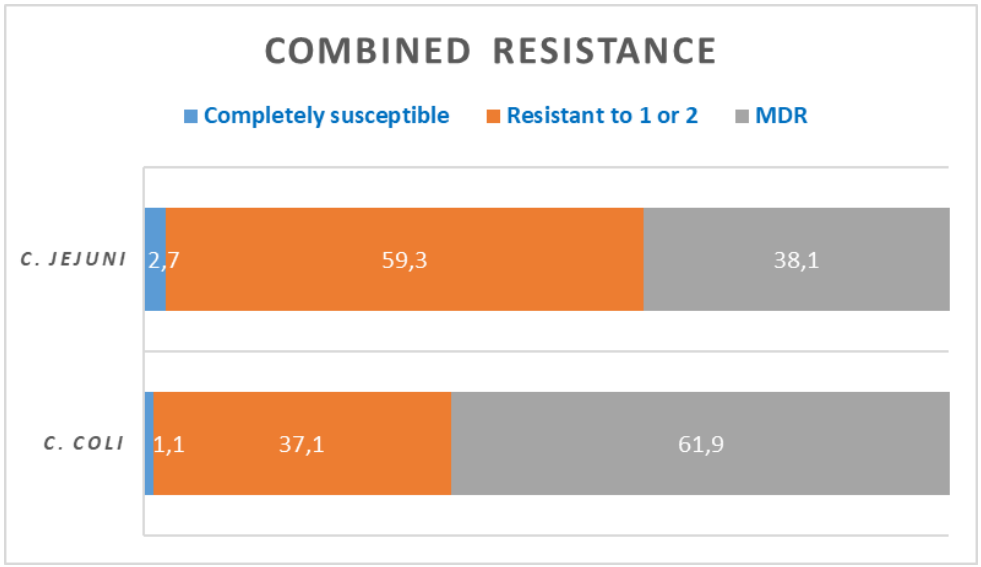
Resistance to erythromycin:

The proportion of resistant isolates is significantly higher in *C. coli* (52.2%) than in *C. jejuni* (3.3%), P-value <0.001.

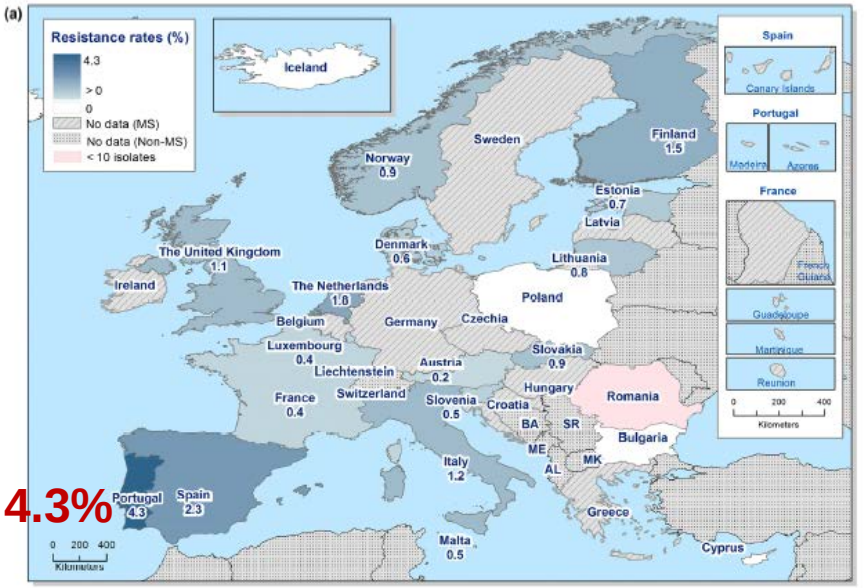
For both species, the proportion of ERY-resistance in Portugal is much higher than the EU median for human isolates: *C. jejuni* (3.3% vs 1.5%), *C. coli* (52.2% - 12.9%), in 2018-2019[1]

1- EFSA and ECDC 2021. The European Union Summary Report on Antimicrobial Resistance in zoonotic and indicator bacteria from humans, animals and food in 2018/2019. EFSA Journal 2021;19(4):6490, 179 pp. https://doi.org/10.2903/j.efsa.2021.6490

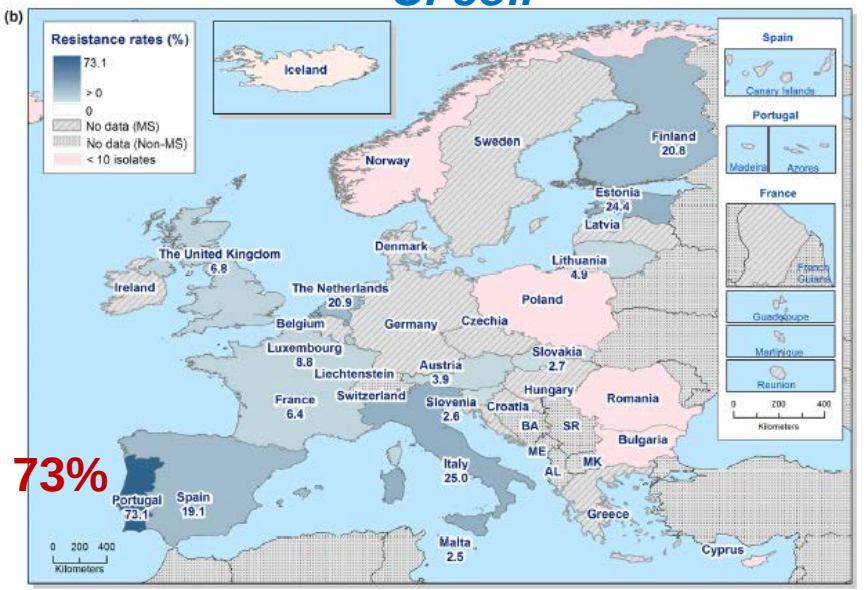

COMBINED RESISTANCE
Completely susceptible
Resistant to 1 or 2
MDR
C. JEJUNI
2,7
59,3
38,1
C. COLI
1,1
37,1
61,9



C. jejuni



C. coli



Combined resistance to the critically-important antimicrobials **ciprofloxacin** and **erythromycin** in:

(a) C. jejuni

(b) C. coli isolates from humans, EU, 2019

1- EFSA and ECDC 2021. The European Union Summary Report on Antimicrobial Resistance in zoonotic and indicator bacteria from humans, animals and food in 2018/2019. EFSA Journal 2021;19(4):6490, 179 pp. https://doi.org/10.2903/j.efsa.2021.6490

Campylobacter in animals/food, PT, erythromycin resistance:

	2015		2016		2017		2018		2020	
	C. jejuni	*C. coli*	*C. jejuni*	*C. coli*	*C. jejuni*	*C. coli*	*C. jejuni*	*C. coli*	*C. jejuni*	*C. coli*
Broilers			**10.4% (n=67)**				**16.4%**		**6.4%**	
Broilers meat	**25% (n=5)**	**40% (n=15)**								
Fattenning turkeys			**11.1% (n=36)**				**23.1%**		**16.2%**	
Pig meat				**33.3% (n=3)**		**60% (n=5)**				

Data reported on Campylobacter resistance in animals and food in PT is still limited.

C. coli tends to be more resistant than *C. jejuni*.

C. coli isolates from pig meat are more resistant than from broilers meat.

Combined **resistance to ciprofloxacin and erythromycin, *Campylobacter jejuni***

Broilers, 2018

Broilers, 2020

Fattening turkeys, 2018

Fattening turkeys, 2020

ECDC&EFSA reports, 2021, 2022

The problem of macrolides resistance

For both species, the proportion of **ERY-resistant isolates in Portugal is much higher than the EU median**.

But the proportion of resistant isolates between *C. coli* and *C. jejuni* (**52.2% vs 3.3%, P < 0.001, mean 2015-2021**) is very discrepant.

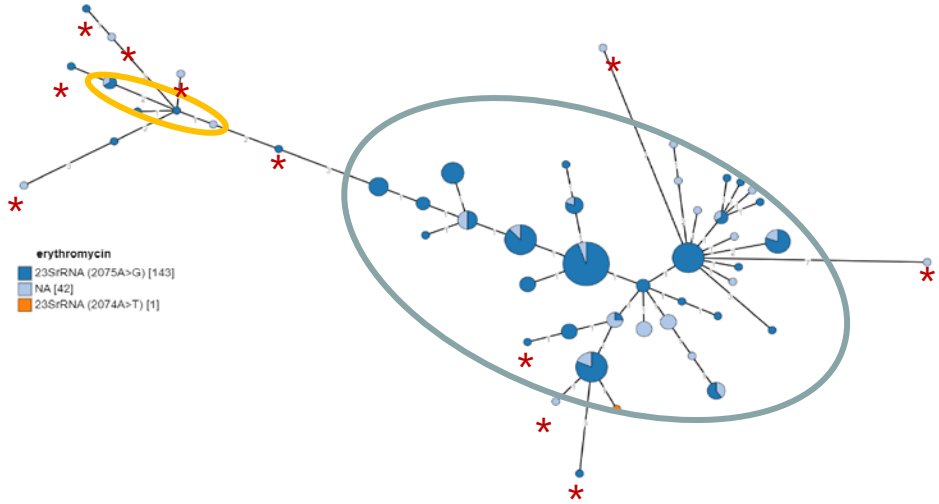
Resistance in animal and food isolates (broilers and pigs) is even higher than in human isolates.

Factors to consider:

- ✓ Dominant and persistent *C. coli* ERY_R lineages circulating over the years
- ✓ Different sources of *Campylobacter* infection (**poultry** vs **pig** vs cattle)
- ✓ Antimicrobial agents consumption in food producing animals (differences?)
- ✓ Different fitness cost

C. coli, n=136 Human, n=50 Poultry

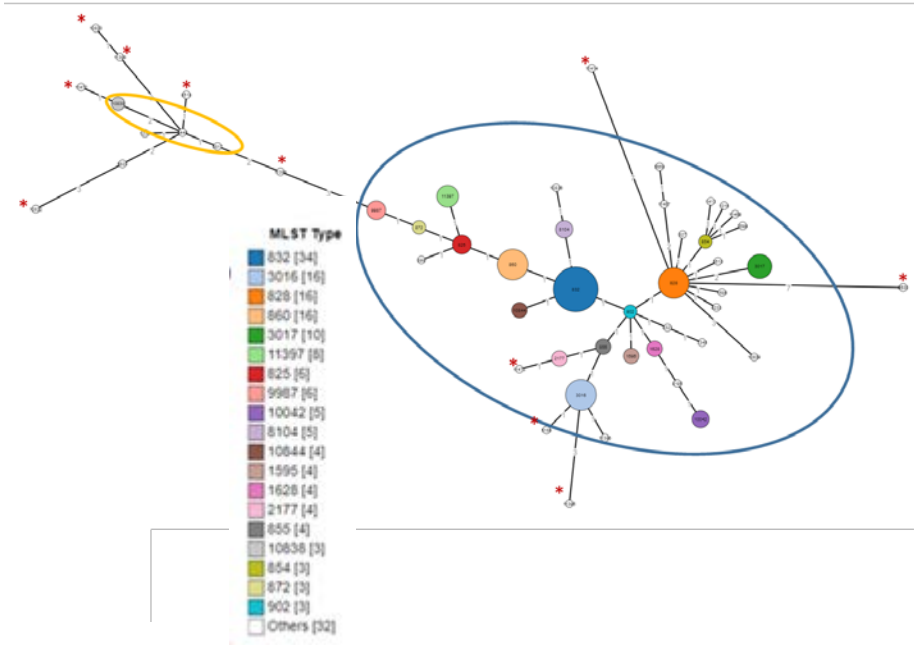
Dominant and persistent ERY_R lineages circulating over the years?

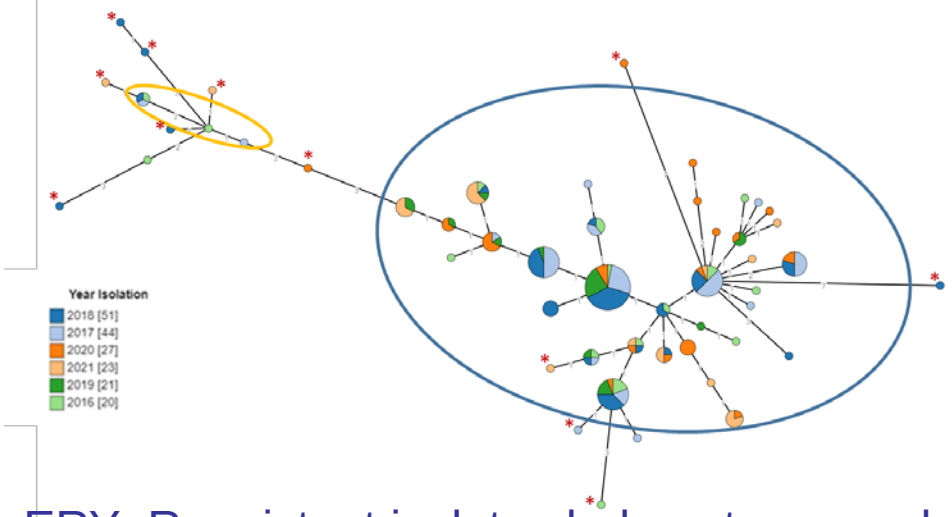


CC-ST828 n=168

CC-ST1150 n=6

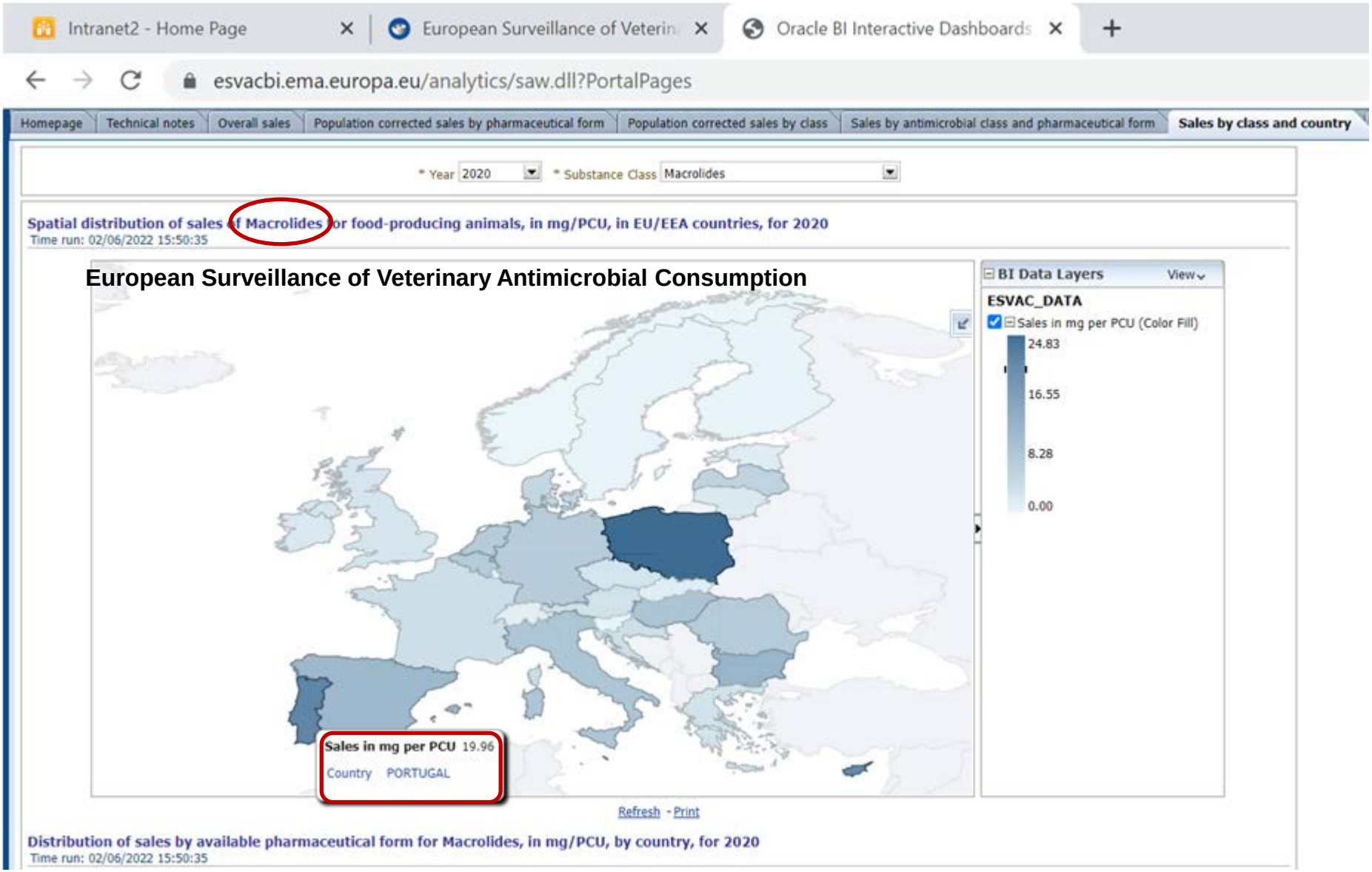
*other CC n=12





ERY_R resistant isolates belong to several different STs that circulate over the studied period (2016-2021).

Antimicrobial agents consumption:



https://esvacbi.ema.europa.eu/analytics/saw.dll?PortalPages

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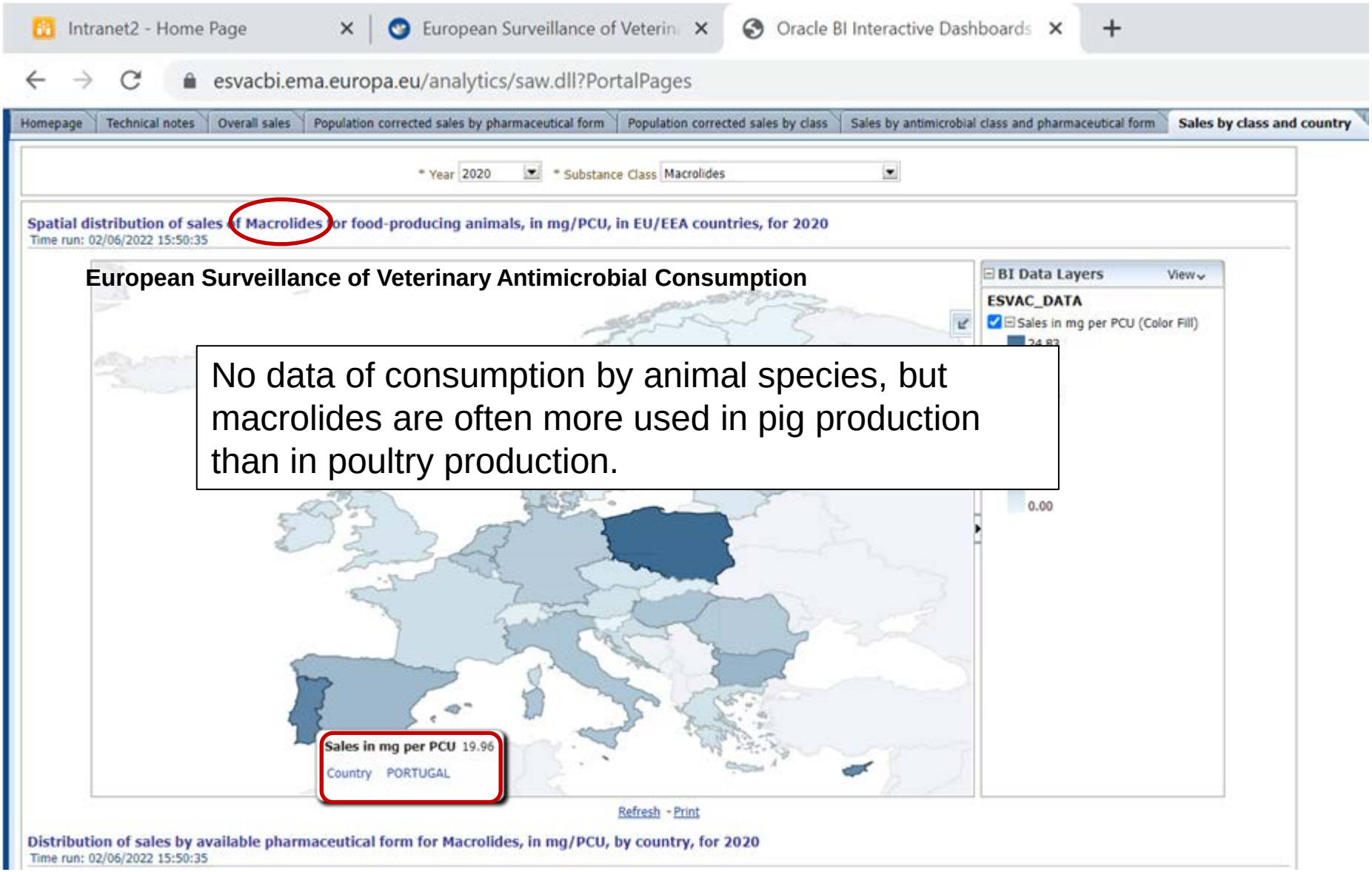


No data of consumption by animal species, but macrolides are often more used in pig production than in poultry production.

https://esvacbi.ema.europa.eu/analytics/saw.dll?PortalPages

The problem of macrolides resistance

Dominant and persistent ERY_R lineages over the years? Not supported by our data.

Different sources of infection for *C. coli*? Considering macrolides consumption in food producing animals, *C. coli* is more frequent in adults than in children, other sources of MDR C. coli should be evaluated under an OH perspective.

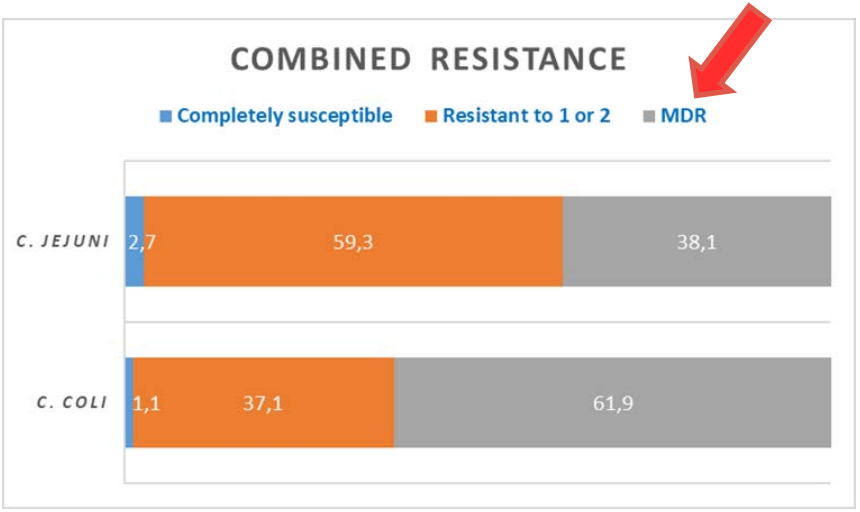
Different fitness cost? Macrolides resistance imposes a fitness cost to the bacterium, so this scenario is UNLIKELY, but diferences between species may exist[1].

[1]Luangtongkum T, et al. *Future Microbiol*. 2009;4(2):189-200.doi:10.2217/17460913.4.2.189

CAMPYLOBACTER RESISTANCE TO CARBAPENEMS

Carbapenems possess the broadest spectrum of activity and greatest potency against Gram-positive and Gram-negative bacteria.

Last resort antibiotics used when patients with infections become gravely ill or are suspected of harbouring resistant bacteria.



When are they used for *Campylobacter*?

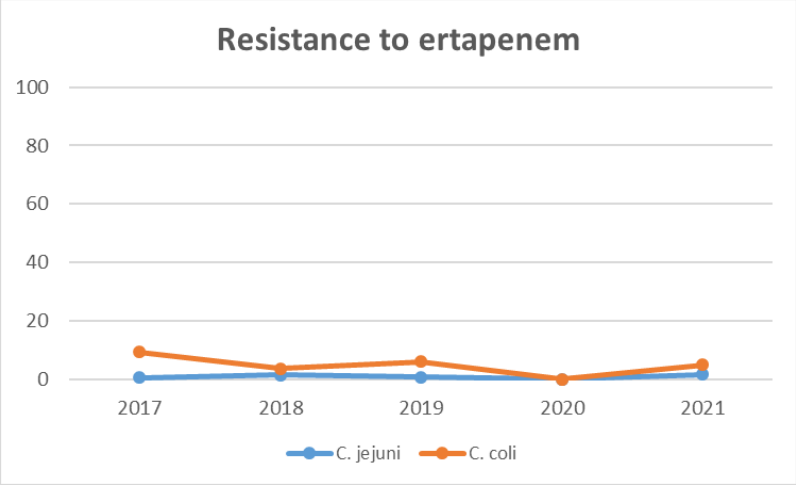
✓ Complicated Campylobacteriosis that does not respond to the first line of treatment

✓ Complicated Campylobacteriosis with a MDR strain.

CAMPYLOBACTER RESISTANCE TO CARBAPENEMS

Resistance of *Campylobacter* to carbapenems is a rare event.

1) **Circulating strains** that are monitored through surveillance, since 2017 (Method: E-test for Ertapenem, CA-SFM 2022, cut-off MIC>1mg/L).



***C. jejuni*: 0.4%-1.5%**

***C. coli*: 3.6%-9.3%**

MICs variation for non-susceptible isolates:

C. coli: **2.0- >32 mg/L**

C. jejuni: **1.5-6 mg/L**

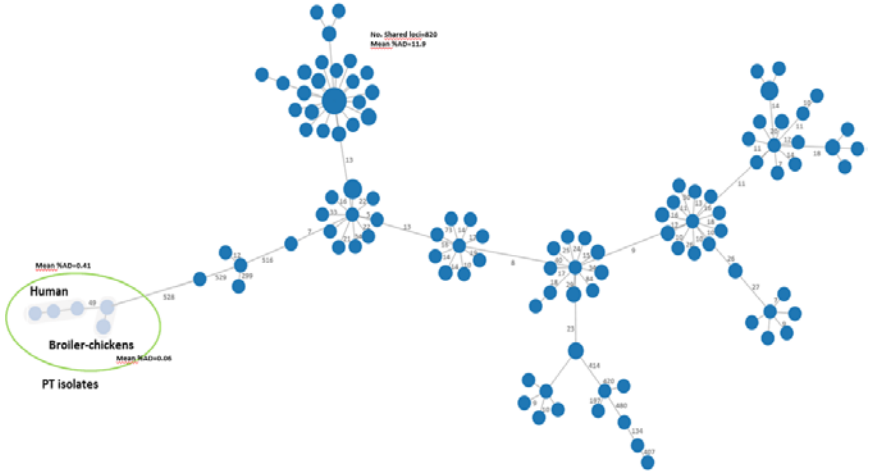
Susceptible isolates: MICs 0.016-0.75 mg/L

1) Circulating strains

A genomic-based epidemiological study of MDR *Campylobacter* isolated from humans and poultry

SPECIES	ISOLATE ID	ORIGIN	COUNTRY/YEAR OF ISOLATION	ANTIBIOTIC RESISTANCE PROFILE
Campylobacter coli	3037	Human, non-outbreak	Portugal/2017	Ampicillin R
Campylobacter coli	3038	Human, non-outbreak	Portugal/2017	Ciprofloxacin R
Campylobacter coli	3048	Human, non-outbreak	Portugal/2017	Erythromycin R
Campylobacter coli	C32/1	broiler-chickens, farm A	Portugal/2017	Tetracycline R
Campylobacter coli	P32/1	broiler-chickens, farm A	Portugal/2017	**Ertapenem R**
Campylobacter coli	F13/2	broiler-chickens, farm B	Portugal/2017	Gentamycin S
Campylobacter coli	F13/3	broiler-chickens, farm B	Portugal/2017	**MIC_Ert>32 mg/L**

MIC for Ertapenem >32 mg/L, except for isolates 3037 and 3048 with MIC=16 mg/L



Phylogeny of C. coli ST860 strains based on a dynamic gene-by-gene approach using a wgMLST schema with 2477 loci

Main findings:

All the 7 isolates belonged to ST-860, CC-828

Close phylogenetic proximity of *C. coli* MDR isolates among human and chickens, supporting a bi-directional transmission among these 2 reservoirs and the evidence that humans are a key risk factor for introducing *Campylobacter* contamination in the poultry production environment.

1) Circulating strains

A genomic-based epidemiological study of MDR Campylobacter isolated from humans and poultry

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Campylobacter coli	3038	Human, non-outbreak	Portugal/2017	
Campylobacter coli	3048	Human, non-outbreak	Portugal/2017	
Campylobacter coli	C32/1	broiler-chickens, farm A	Portugal/2017	
Campylobacter coli	P32/1	broiler-chickens, farm A	Portugal/2017	
Campylobacter coli	F13/2	broiler-chickens, farm B	Portugal/2017	
Campylobacter coli	F13/3	broiler-chickens, farm B	Portugal/2017	

MIC for Ertapenem >32 mg/L, except for isolates 3037 and 3048 with MIC=16 mg/L

Main findings:

All the 7 isolates belonged to ST-860, CC-828; all harbour a very rare (0.1%) ***porA* PubMLST allele 8**.

Close-proximity among PT human isolates: **10 core-CDS SNPs**, **1 SNP was found in the *cmeR* gene** (transcriptional repressor of efflux pump CmeABC) (Isolate 3038, MIC>32 mg/L)

PRODUCT	Alteration
Transcriptional repressor of CmeABC operon, CmeR	missense_variant c.128C>T p.Ser43Leu

1) Circulating strains

A genomic-based epidemiological study of MDR *Campylobacter coli* isolated from humans and poultry

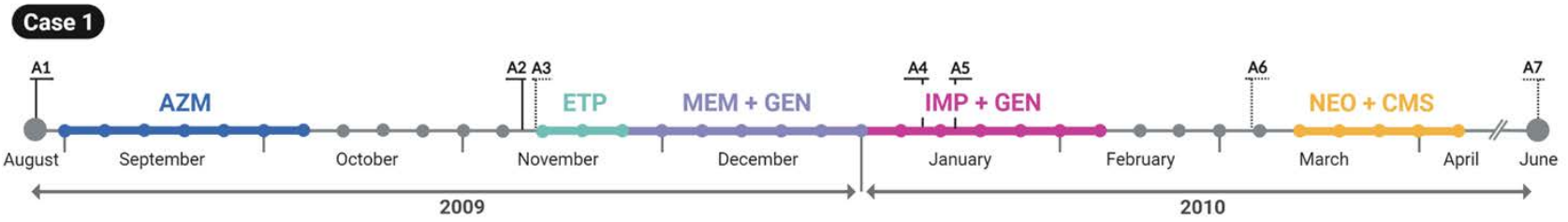
SPECIES	ISOLATE ID	ORIGIN	COUNTRY/YEAR OF ISOLATION	ANTIBIOTIC RESISTANCE PROFILE
Campylobacter coli	3037	Human, non-outbreak	Portugal/2017	Ampicillin R; Ciprofloxacin R; Erythromycin R; Tetracycline R; **Ertapenem R**; Gentamycin S — *MIC for Ertapenem >32 mg/L, except for isolates 3037 and 3048 with MIC=16 mg/L*
Campylobacter coli	3038	Human, non-outbreak	Portugal/2017	
Campylobacter coli	3048	Human, non-outbreak	Portugal/2017	
Campylobacter coli	C32/1	broiler-chickens, farm A	Portugal/2017	
Campylobacter coli	P32/1	broiler-chickens, farm A	Portugal/2017	
Campylobacter coli	F13/2	broiler-chickens, farm B	Portugal/2017	
Campylobacter coli	F13/3	broiler-chickens, farm B	Portugal/2017	**MIC_Ert>32 mg/L**

⇒ **SNPs within *porA* (porin function) and/or *cmeABC* operon (EP overexpression) are likely associated with resistance to ertapenem.**

[1]Clin. Microbiol. Rev. 2012, 25(4):661. DOI:https://doi.org/10.1128/CMR.00043-12

CAMPYLOBACTER RESISTANCE TO CARBAPENEMS

1) ***In vivo* selection** of resistance to carbapenems



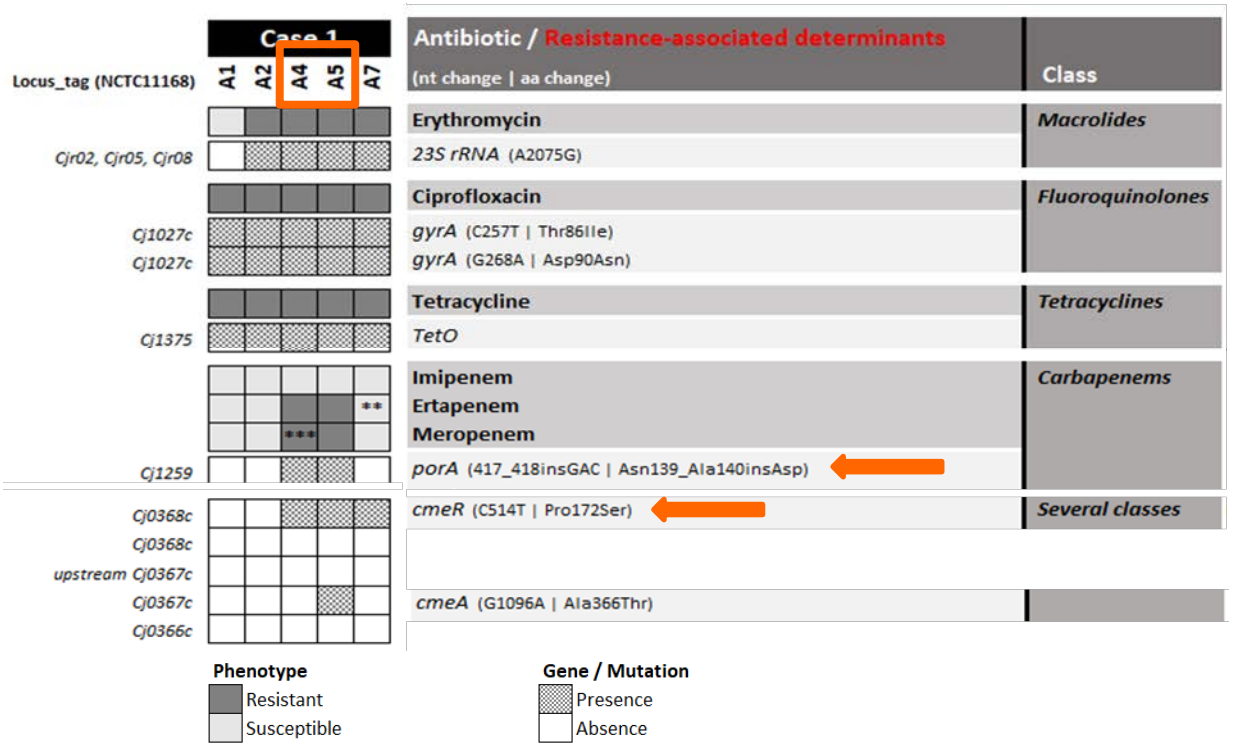
Strain_Id (for the poster)	MLST_ST (Clonal Complex)	Biological sample (Isolation source)	Isolation date	Antibiotic treatment	Antimicrobial resistance						
					Ery	Cip	Tet	Amx	Ert	Mer	Imp
A1	1709 (ST-1034 complex)	Blood	08-26-2009	Azythromycin (6 weeks)	S	R	R	R	0.19	0.047	0.032
A2		Blood	11-11-2009	Ertapenem (12 days)	R	R	R	R	0.125	0.064	0.032
A3		Stool	11-13-2009	Meropenem (6 weeks) + Gentamicin	R	R	R	R	0.125	0.064	0.032
A4		Blood	01-12-2010		R	R	R	R	>32	4-8	0.38
A5		Blood	01-18-2010	Imipenem (6 weeks) + Gentamicin	R	R	R	R	>32	>32	0.19
A6		Stool	03-05-2010	Neomycin (4 weeks)	R	R	R	R	>32	4-8	0.25
A7		Stool	06-15-2010	Colimycin (4 weeks)	R	R	R	R	1	0.094	0.094

Case 1: patient with congenital agammaglobulinemia; *C. jejuni* was cultured from blood and stool samples. Two months into treatment with azithromycin of a macrolide susceptible strain, a macrolide-resistant strain was isolated from blood. Switching to ertapenem, resulted in a relapse with a carbapenem-resistant strain (high level).

Manuscript in preparation

CAMPYLOBACTER RESISTANCE TO CARBAPENEMS

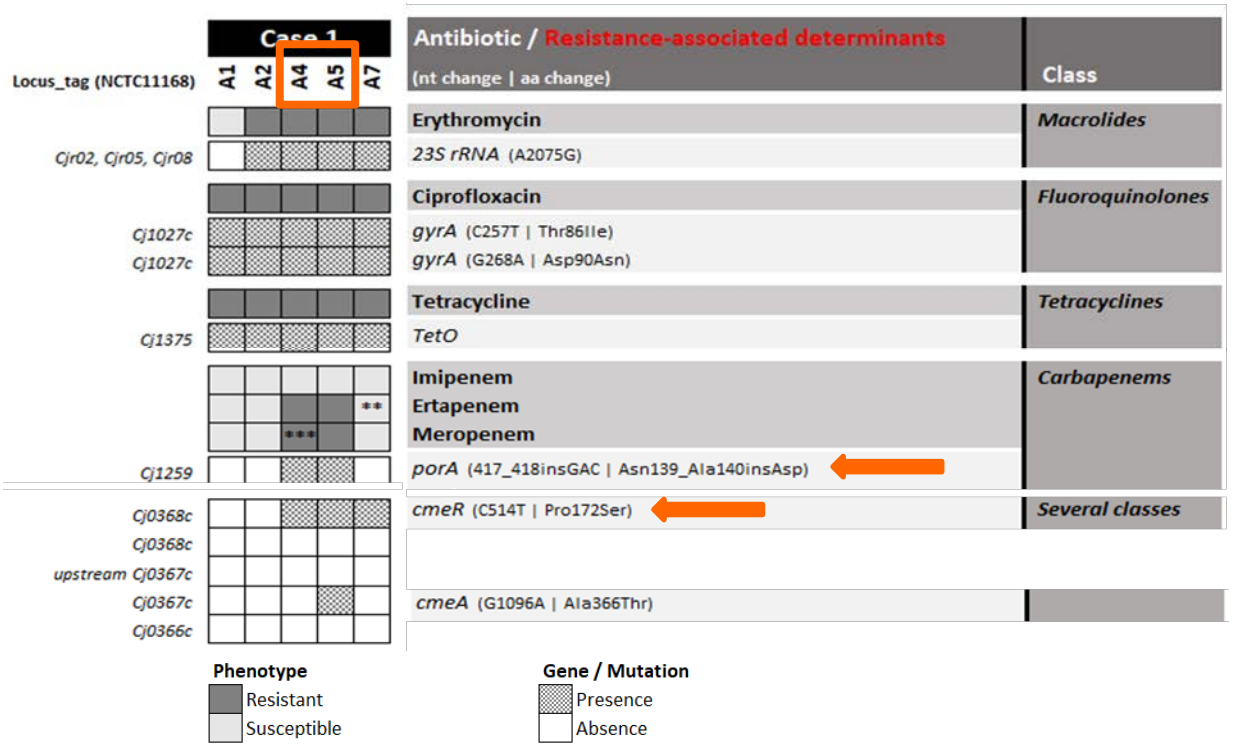
1) ***In vivo* selection** of resistance to carbapenems

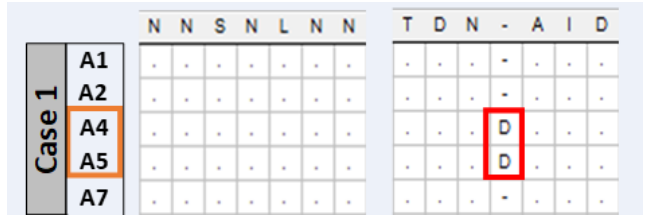


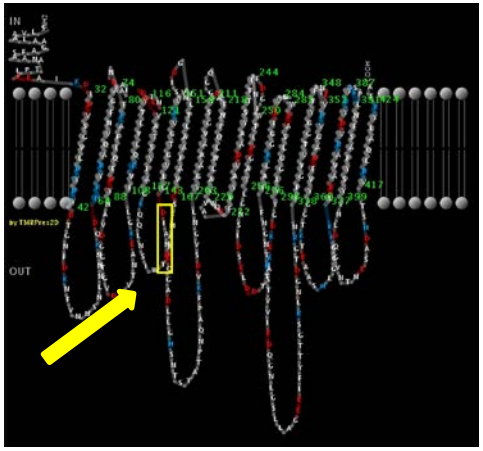
CAMPYLOBACTER RESISTANCE TO CARBAPENEMS

1) ***In vivo* selection** of resistance to carbapenems

PorA MOMP







Altered size and charge of the pore, resulting in decreased susceptibility to β-lactams[1].

[1]Clin. Microbiol. Rev. 2012, 25(4):661. DOI:https://doi.org/10.1128/CMR.00043-12

CONCLUSIONS AND MAIN MESSAGES

- ✓ Importance of *C. coli* infections (represents 10.8% to 12.6% of human infections in Portugal)
- ✓ Resistance to antimicrobials regarded as critically important for clinical treatment is a larger problem in *C. coli* than in *C. jejuni*
- ✓ A better knowledge of the resistance levels of *C. coli*, not only in poultry, but also in pigs and ruminants is necessary
- ✓ More source attribution studies for MDR *C. coli* at country level are necessary
- ✓ More efforts to reduce the burden of MDR *C. coli* (reduce the use of antibiotics in food producing animals) – One Health perspective.

CONCLUSIONS AND MAIN MESSAGES

- ✓ Carbapenem (ertapenem) resistance in *Campylobacter* is a rare event but strains with diferent levels of resistance are already circulating in patients and in animals, and should be monitored.
- ✓ SNPs within *porA* and/or *cmeABC* EF are likely associated with resistance to carbapenems in *Campylobacter*.
- ✓ More studies are needed to unveil resistance determinants, specially associated with low-level resistance.

THANK YOU FOR YOUR ATTENTION

Mónica Oleastro

monica.oleastro@insa.min-saude.pt

https://www.researchgate.net/profile/Monica_Oleastro

ORCID: orcid.org/0000-0001-6360-2576

NRL, PT

Mónica Oleastro
Frederico Alves
Alexandra Nunes

Collaborative Centre

Prof. Philippe Lehours-Keisler
CNR des Campylobacters et des Hélicobacters
(www.cnrch.fr)