Istituto Zooprofilattico Sperimentale
dell'Umbria e delle Marche "Togo Rosati"

Antibiotic resistance in the aquatic environment and in animals

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Regional Workshop for WOAH National Focal Points for Aquatic Animals.
Chioggia, October 19, 2023

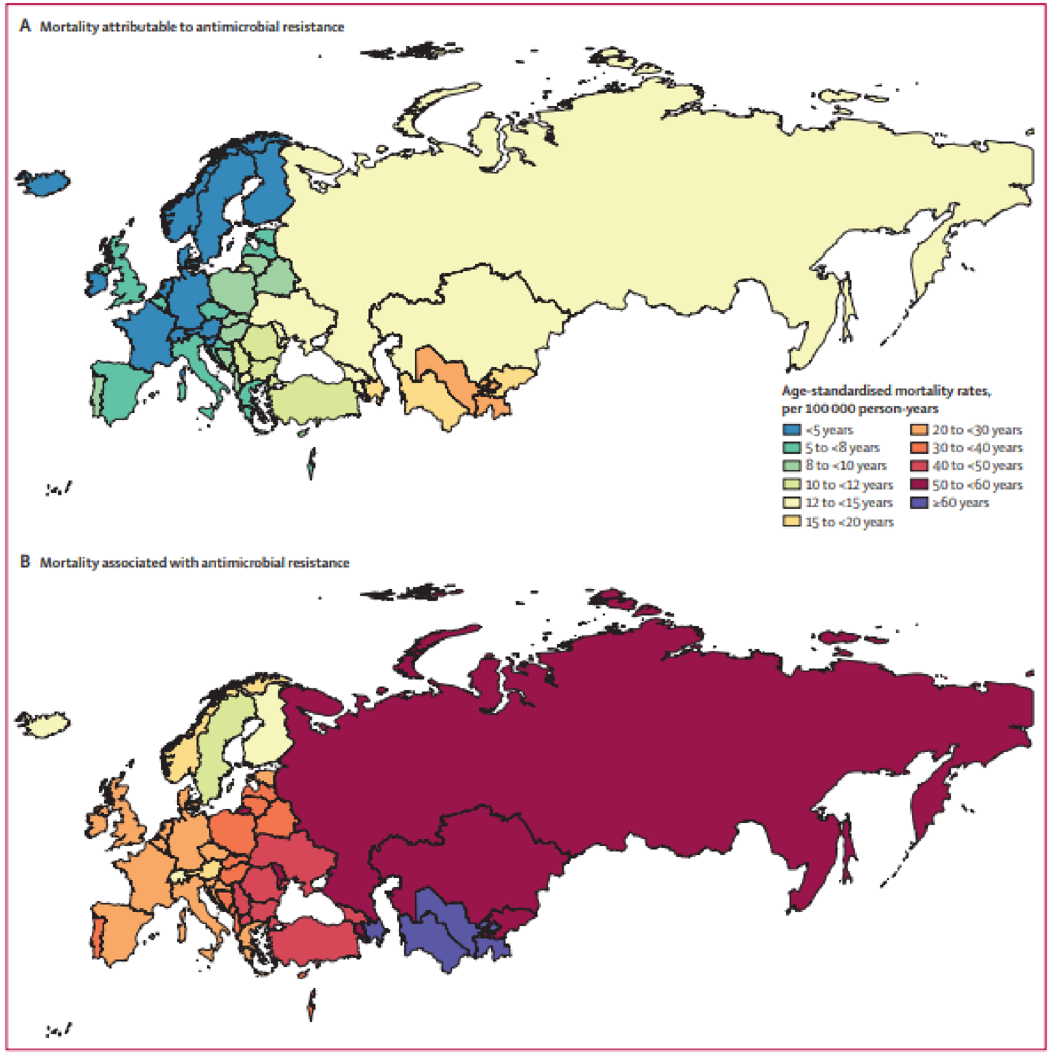


Figure 3: Cross-country comparison of age-standardised mortality rates per 100 000 person-years for deaths attributable to (A) and associated with (B) antimicrobial resistance in the WHO European region in 2019

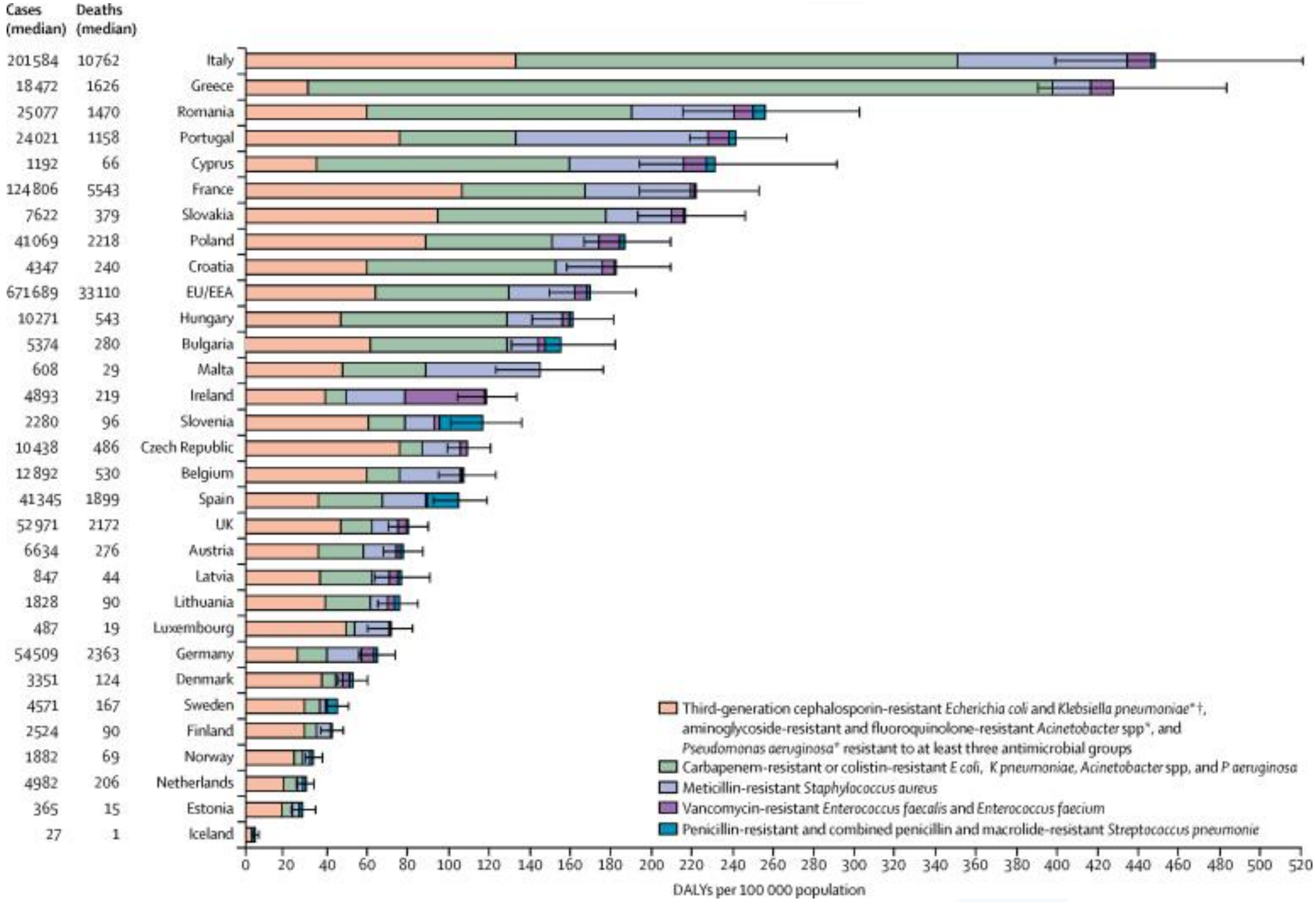
AMR is one the first 10 issues for public health worldwide (WHO, 2021)

4.95 milions (3·62–6·57) of deaths were associated with AMR in 2019

Resistance to fluoroquinolones and beta-lactams alone is responsible of more than 70% of deaths associated to AMR*

*Naghavi, *et al*. *Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis*. Lancet, 2022.
**Antimicrobial Resistance Collaborators. *The burden of bacterial antimicrobial resistance in the WHO European region in 2019: a cross-country systematic analysis.* Lancet Public Health, 2022.

Cassini A, *et al. Attributable deaths and disability-adjusted life-years caused by infections with antibiotic-resistant bacteria in the EU and the European Economic Area in 2015: a population-level modelling analysis.* Lancet Infect Dis. 2019.



AMR: the main facts

- The lack of biosecurity allows the spread of bacteria, included the antibiotic-resistant ones

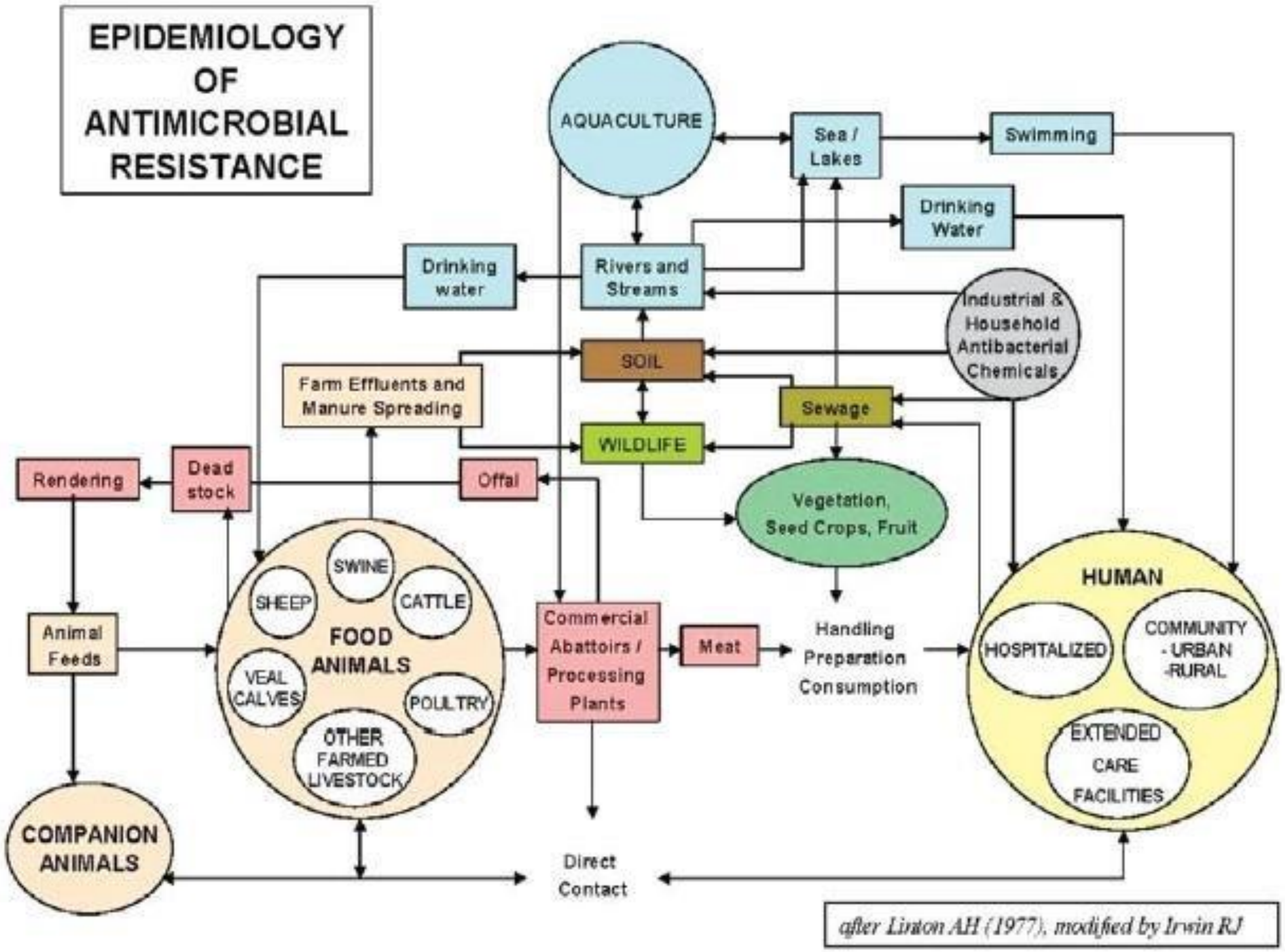
- Antibiotic-resistance is associated with relevant economic costs for the public health systems

- The main risk factor for AMR is antibiotic use

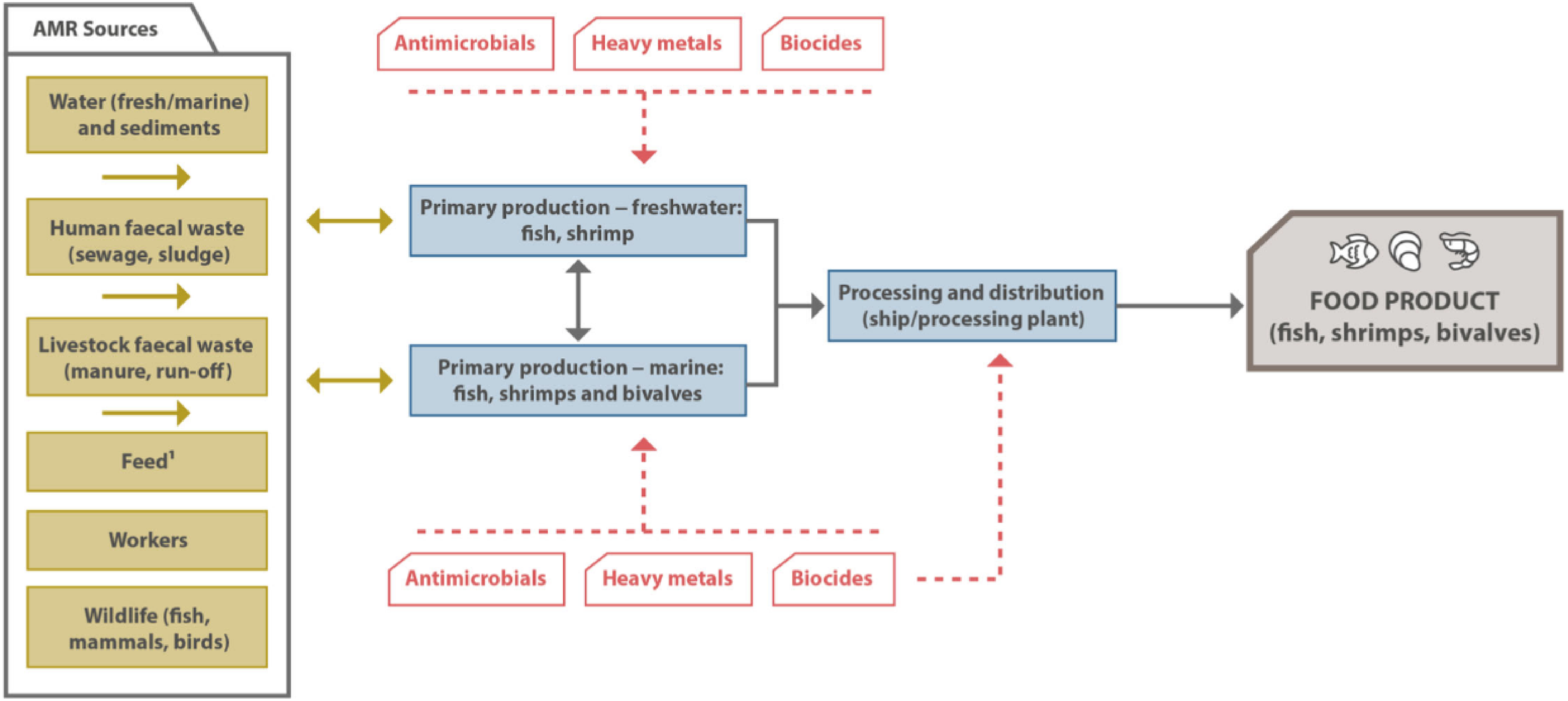
Table 6. Estimated PCU (in 1,000 tonnes) of the population of food-producing animals[1,2], by country, in 2020

Country	Cattle	Pigs	Poultry	Sheep and goats	Fish	Rabbits	Horses	Total
Austria	422	357	89	36	0	0	38	942
Belgium	484	832	285	19	0	4	122	1,745
Bulgaria	114	76	42	100	14	<0.01	23	368
Croatia	103	92	49	52	21	0.01	11	329
Cyprus	20	45	13	43	0	0.1	2	123
Czechia	288	199	131	16	20	7	39	699
Denmark	380	1,754	123	12	46	0	70	2,385
Estonia	59	43	2	5	1	0	5	116
Finland	207	146	85	12	15	0	30	494
France	3,065	1,811	1,087	644	46	39	272	6,965
Germany	2,922	3,534	1,022	132	19	23	520	8,173
Greece	77	103	144	759	129	2	2	1,217
Hungary	152	315	211	82	9	8	24	801
Iceland	19	6	6	41	41	0	23	135
Ireland	1,304	294	111	344	38	0	100	2,190
Italy	1,424	782	766	571	59	30	157	3,790

European Medicines Agency, European Surveillance of Veterinary Antimicrobial Consumption, 2021. 'Sales of veterinary antimicrobial agents in 31 European countries in 2019 and 2020'. (EMA/58183/2021).


EPIDEMIOLOGY OF ANTIMICROBIAL RESISTANCE
AQUACULTURE
Sea / Lakes
Swimming
Drinking Water
Drinking water
Rivers and Streams
Industrial & Household Antibacterial Chemicals
SOIL
Farm Effluents and Manure Spreading
Sewage
WILDLIFE
Rendering
Dead stock
Offal
Vegetation, Seed Crops, Fruit
SWINE
SHEEP
CATTLE
FOOD ANIMALS
VEAL CALVES
POULTRY
OTHER FARMED LIVESTOCK
Animal Feeds
Commercial Abattoirs / Processing Plants
Meat
Handling Preparation Consumption
HUMAN
HOSPITALIZED
COMMUNITY - URBAN -RURAL
EXTENDED CARE FACILITIES
COMPANION ANIMALS
Direct Contact
after Linton AH (1977), modified by Irwin RJ

Epidemiology of AMR: aquaculture



EFSA BIOHAZ Panel, 2021. Scientific Opinion on the role played by the environment in the emergence and spread of antimicrobialresistance (AMR) through the food chain. EFSA Journal.

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Epidemiology of AMR: aquaculture

Overall, the amount of antibiotics used in aquaculture in EU is limited compared to other animal production systems.

However, since antibiotics are released directly in water, they can generate a significant selective pressure for AMR, higher than the one realeased by other production systems or human settlements.

EFSA BIOHAZ Panel, 2021. ScientificOpinion on the role played by the environment in the emergence and spread of antimicrobialresistance (AMR) through the food chain. EFSA Journal 2021;19(6):6651, 188 pp.https://doi.org/10.2903/j.efsa.2021.6651

AMR in seafood

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Occurrence and temporal distribution of extended-spectrum β-lactamase-producing *Escherichia coli* in clams from the Central Adriatic, Italy

Francesca Leoni[1*], Luca Sacchini[1], Silvia Pieralisi[1], Gabriele Angelico[1], Chiara Francesca Magistrali[2], Lucilla Cucco[2], Francesca Romana Massacci[2], Elisa Albini[2], Anna Duranti[2], Cesare Cammà[3], Barbara Secondini[3], Antonio Rinaldi[3] and Francesca Barchiesi[4]

ESBL-producing *E. coli* were isolated from 3% clams

The majority (8/11) of the ESBL-producing *E. coli* isolates were multidrug resistant

ESBL-producing *E. coli* isolates were significantly more commonly recovered in samples with higher *E. coli* levels (14%) than in those with lower levels of *E. coli* (2%).

ID	Source	Beta-lactamase profile	Phenotypic resistance	Beta-lactams	Quinolones/ floroquinolones	Tetracyclines	Aminoglycosides	Sulfonamydes	Phenicols and trimethoprim	Polimixins	Rifamicin	Phosphonics
9449	Salmon	AmpC	AMP FOT CIP TAZ NAL TET GEN FOX ETP SMX TMP FEP	*bla*$_{OXA-1}$ *bla*$_{DHA-1}$ *bla*$_{ACT-16}$ *bla*$_{TEM-1B}$	*qnrB4* *aac(6')-Ib-cr*	*tet(D)*	*aac(6')-Ib-cr* *aac(6')-IIc* *aph(6)-Id* *aadA1* *aph(3'')-Ib* *aadA1*	*sul1*	*catB3* *dfrA1* *dfrA19*	*mcr-9*	*ARR-3*	*fosA*
39742	Clams	AmpC	AMP, FOT, CIP, TAZ, NAL, TMP, TET, GEN, SMX, FOX, ETP, FEP	*bla*$_{OXA-1}$ *bla*$_{DHA-1}$ *bla*$_{ACT-16}$ *bla*$_{TEM-1B}$	*qnrB4* *aac(6')-Ib-cr*	*tet(D)*	*aac(6')-Ib-cr* *aac(6')-IIc* *aph(6)-Id* *aadA1* *aph(3'')-Ib* *aadA1*	*sul1*	*catB3* *dfrA1* *dfrA19*	*mcr-9*	*ARR-3*	*fosA*

Michelacci V, Pieralisi S, Albini E, Massacci FR, Chiani P, Marra M, Carollo M, Magistrali CF, Leoni F *Identificazione di un plasmide che veicola geni di antibiotico-resistenza, incluso il gene mcr-9, in ceppi di* ***Enterobacter hormaechei*** *isolati da prodotti ittici al dettaglio.* Atti SIDILV, 2023

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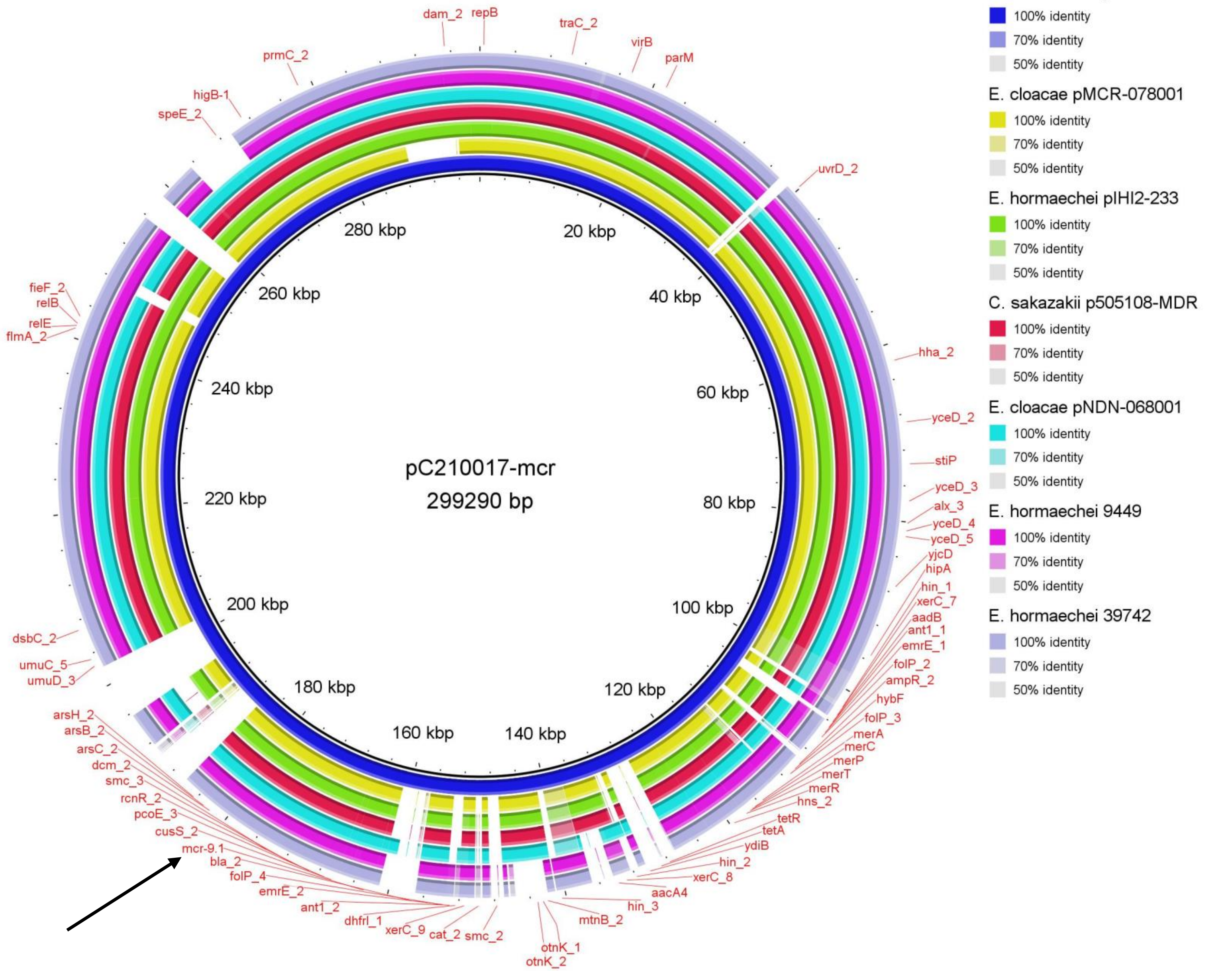

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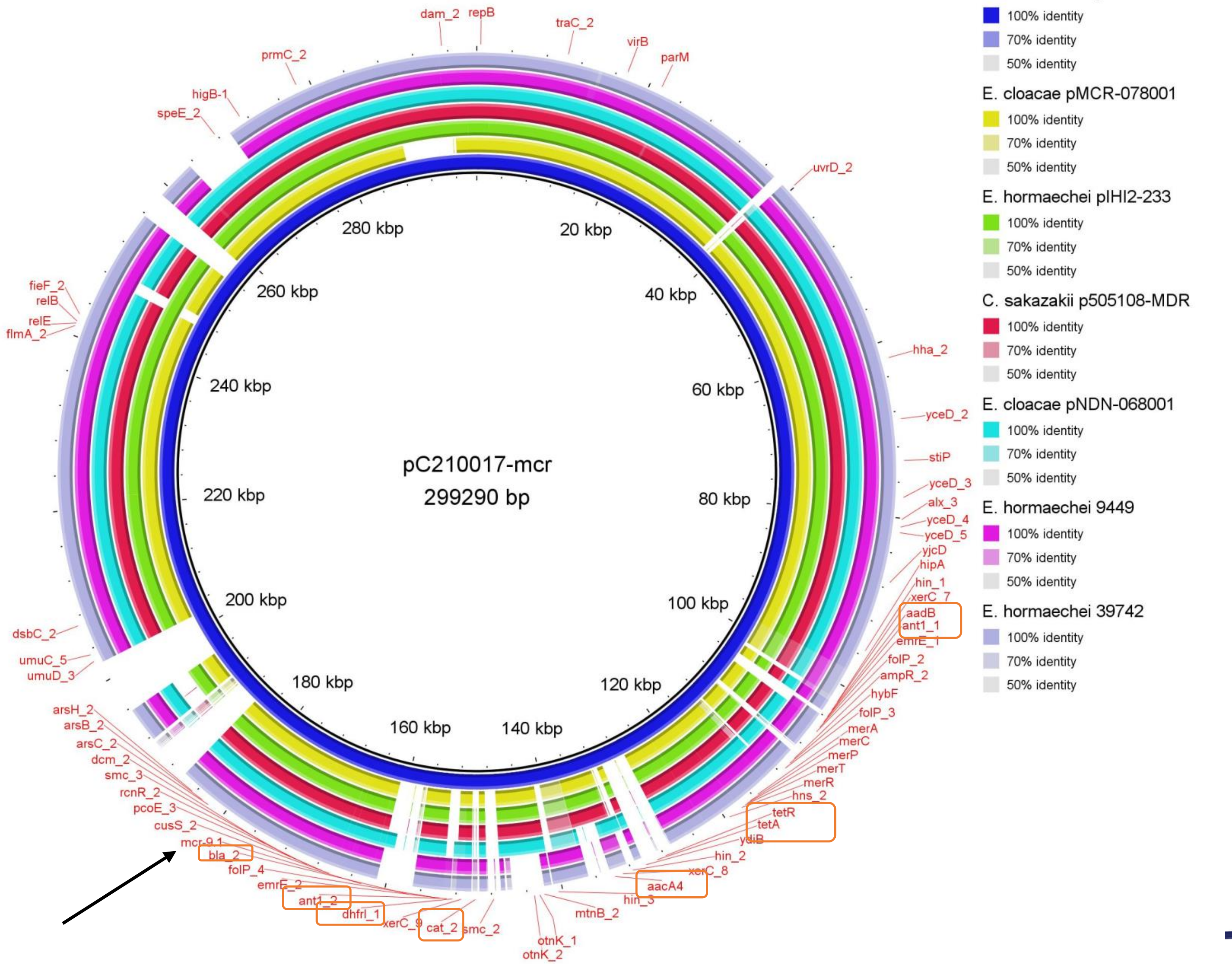
Possibility of horizontal transfer of ARGs from bacteria in aquatic environments to related human pathogens



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Epidemiology of AMR: seafood

Growing market, increased proportion of seafood from aquaculture

Under-appreciated route for transmission

Aquatic food animal supply chains are highly globalized

Aquatic animal products are more likely to be consumed raw

ARTICLE

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https://doi.org/10.1038/s41467-021-25655-8 OPEN

Twenty-year trends in antimicrobial resistance from aquaculture and fisheries in Asia

Daniel Schar [1✉], Cheng Zhao [2], Yu Wang [2], D. G. Joakim Larsson [3,4], Marius Gilbert [1,5,7] & Thomas P. Van Boeckel [2,6,7✉]

ARTICLE

https://doi.org/10.1038/s41467-020-15735-6 OPEN

Aquaculture at the crossroads of global warming and antimicrobial resistance

Miriam Reverter[1,2✉], Samira Sarter[1,3], Domenico Caruso[1], Jean-Christophe Avarre[1], Marine Combe[1], Elodie Pepey[1,3], Laurent Pouyaud[1], Sarahi Vega-Heredía[1], Hugues de Verdal[1,3] & Rodolphe E. Gozlan[1✉]

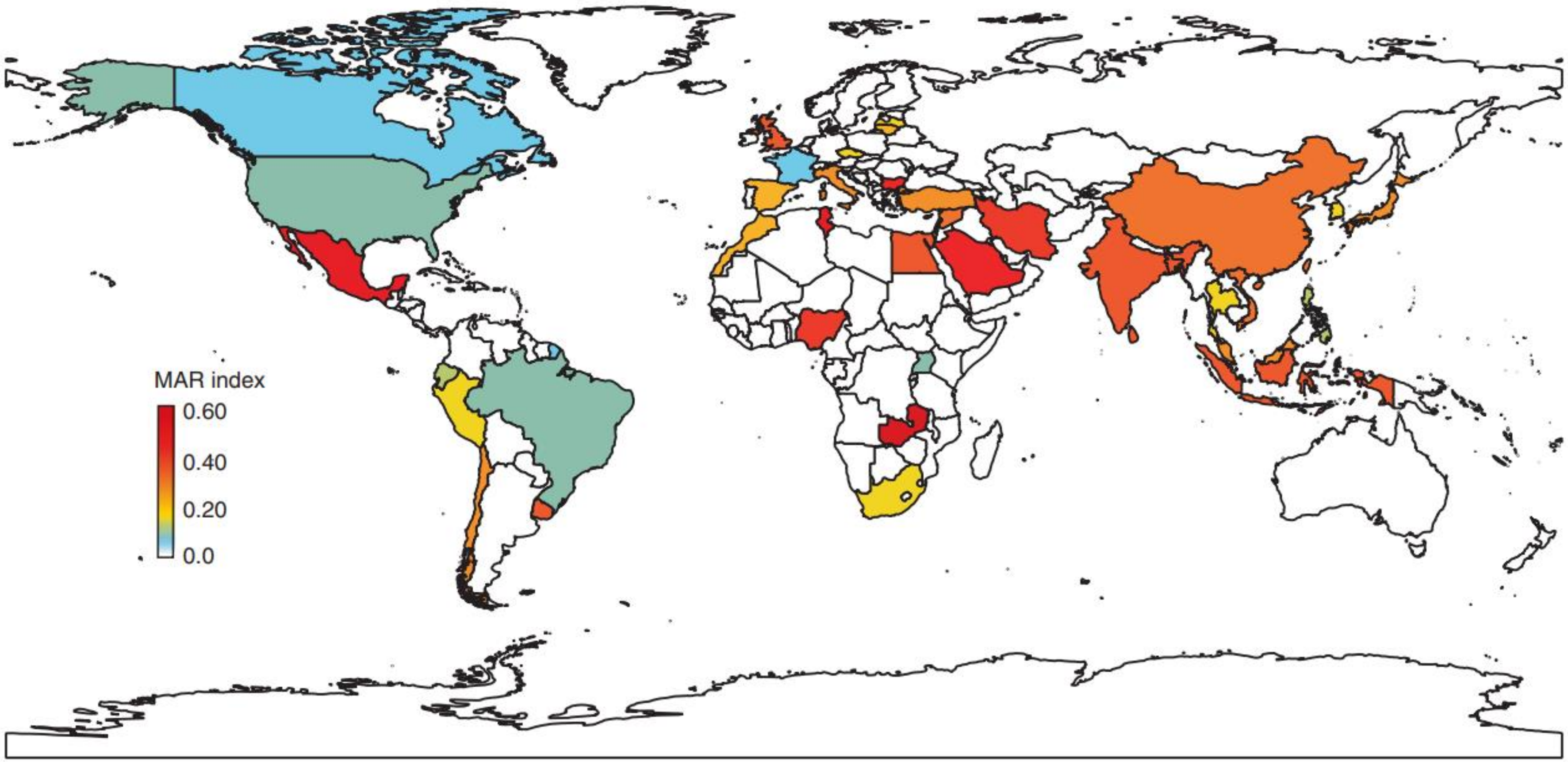

Fig. 2 Global multi-antibiotic resistance (MAR) index calculated from aquaculture-derived bacteria. No MAR index was calculated for countries in white due to data deficiency.

ARTICLE

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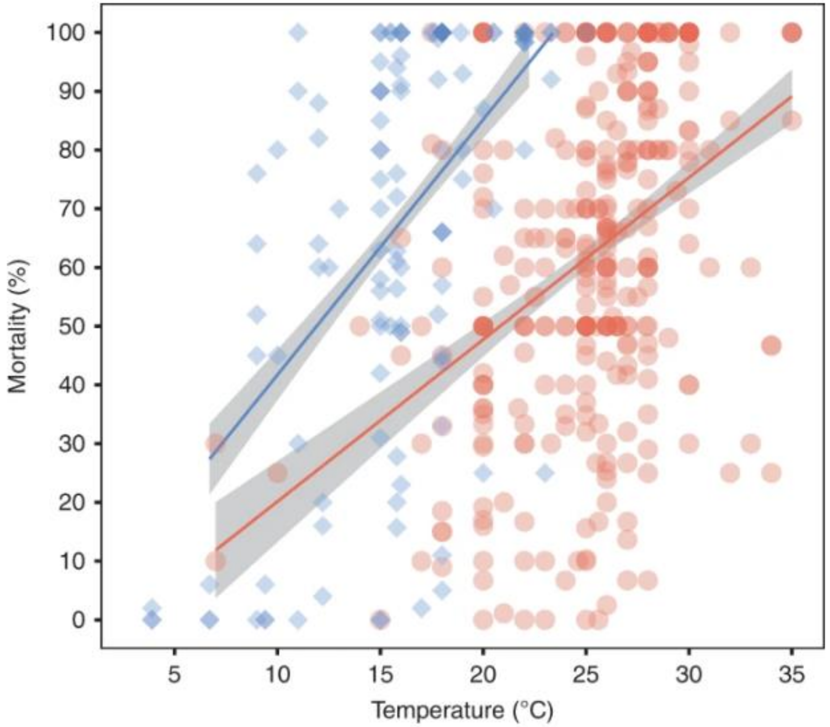
Fig. 1: Predicted changes in mortality (%) of reared aquatic animals infected by bacterial diseases in response to temperature (°C).



Bacterial pathogens: *Aeromonas spp.*, *Edwardsiella spp.*, *F. columnare*, *Lactococcus spp.*, *Streptococcus spp.*, *Vibrio spp.*, and *Yersinia spp.* Red indicates tropical and subtropical host species ($n = 329$), blue indicates temperate host species ($n = 129$). Dots represent the raw data and the lines the linear mixed model predictions with SE.

Models predicted that a temperature increase of 1 °C in warm-water and temperate organisms infected with bacteria could lead to increases of mortality of 2.82–4.12% and 3.87–6.00% respectively

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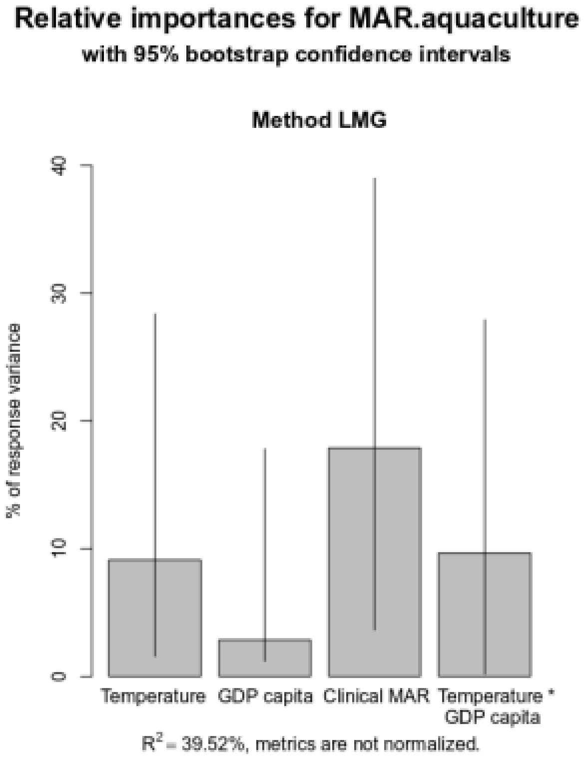

Supplementary figure 2. Relative importance of regressors from the multiple regression model (aquaculture MAR ~ Temperature + GDP capita + Clinical MAR + Temperature*GDP capita) with 95% bootstrap confidence intervals (1000 permutations) assessed by the Lindeman Merenda and Gold (LMG) metrics of the relaimpo R package.

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- Aquaculture environments in most countries present high levels of AMR
- Strong correlation between MAR indices from aquaculture and MAR indices from human clinical bacteria
- The highest AMR levels in aquaculture were observed in economically vulnerable countries
- Higher AMR levels in LMICs can be linked to factors such as poorer sanitation systems or antibiotic misuse

Antibiotic resistance in bivalves

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A systematic review and meta-analysis on antimicrobial resistance in marine bivalves

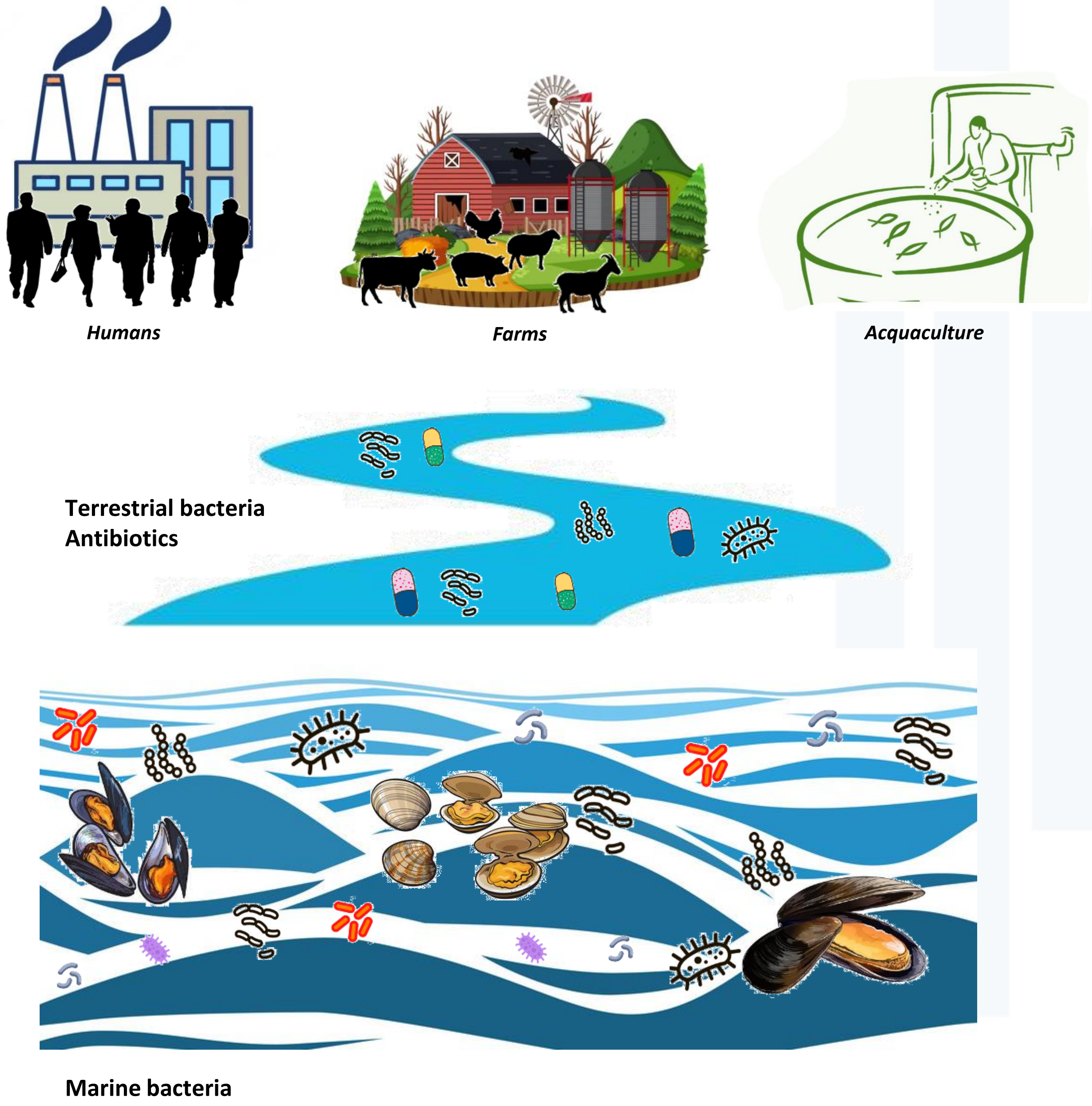
Elisa Albini[1†], Massimiliano Orso[1†], Francesco Cozzolino[2], Luca Sacchini[1], Francesca Leoni[1*] and Chiara Francesca Magistrali[1]

[1]Istituto Zooprofilattico Sperimentale dell'Umbria e Delle Marche 'Togo Rosati', Perugia, Italy,
[2]Health Planning Service, Regional Health Authority of Umbria, Perugia, Italy

Bivalve molluscan shellfish represent an important food commodity

They feed by filtering microalgae from the surrounding waters

Frequently consumed raw or lightly cooked, they can pose a risk for consumers.

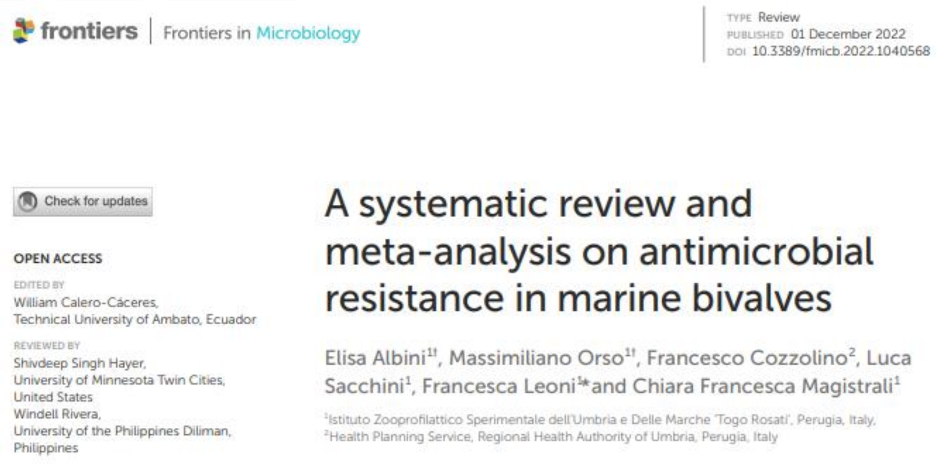

Humans
Farms
Acquaculture
Terrestrial bacteria
Antibiotics
Marine bacteria

Aims

1. What is the prevalence of antibiotic resistance in bacteria from bivalves?

2. Does antibiotic resistance differ in different bacterial species or genera isolated from bivalves?

3. Does AMR vary in bacteria of different geographical origin?



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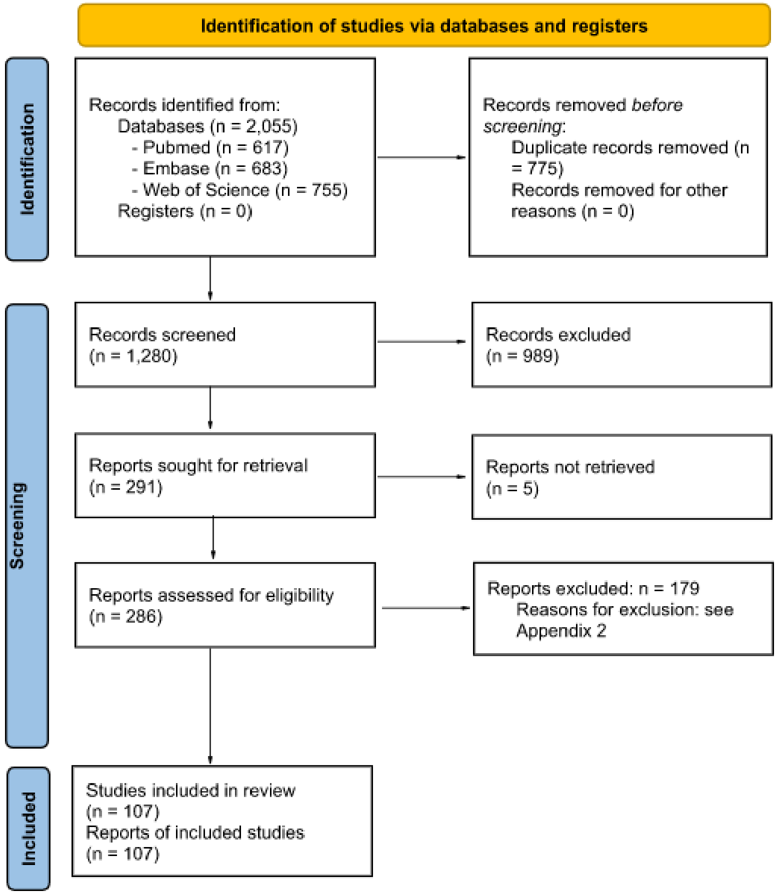
PRISMA 2020 flow diagram for new systematic reviews which included searches of databases and registers only



Bacteria included

Escherichia coli

Salmonella spp.

Terrestrial

Aeromonas spp.

Vibrio spp.

Vibrio parahaemolyticus

Vibrio cholerae

Marine environment

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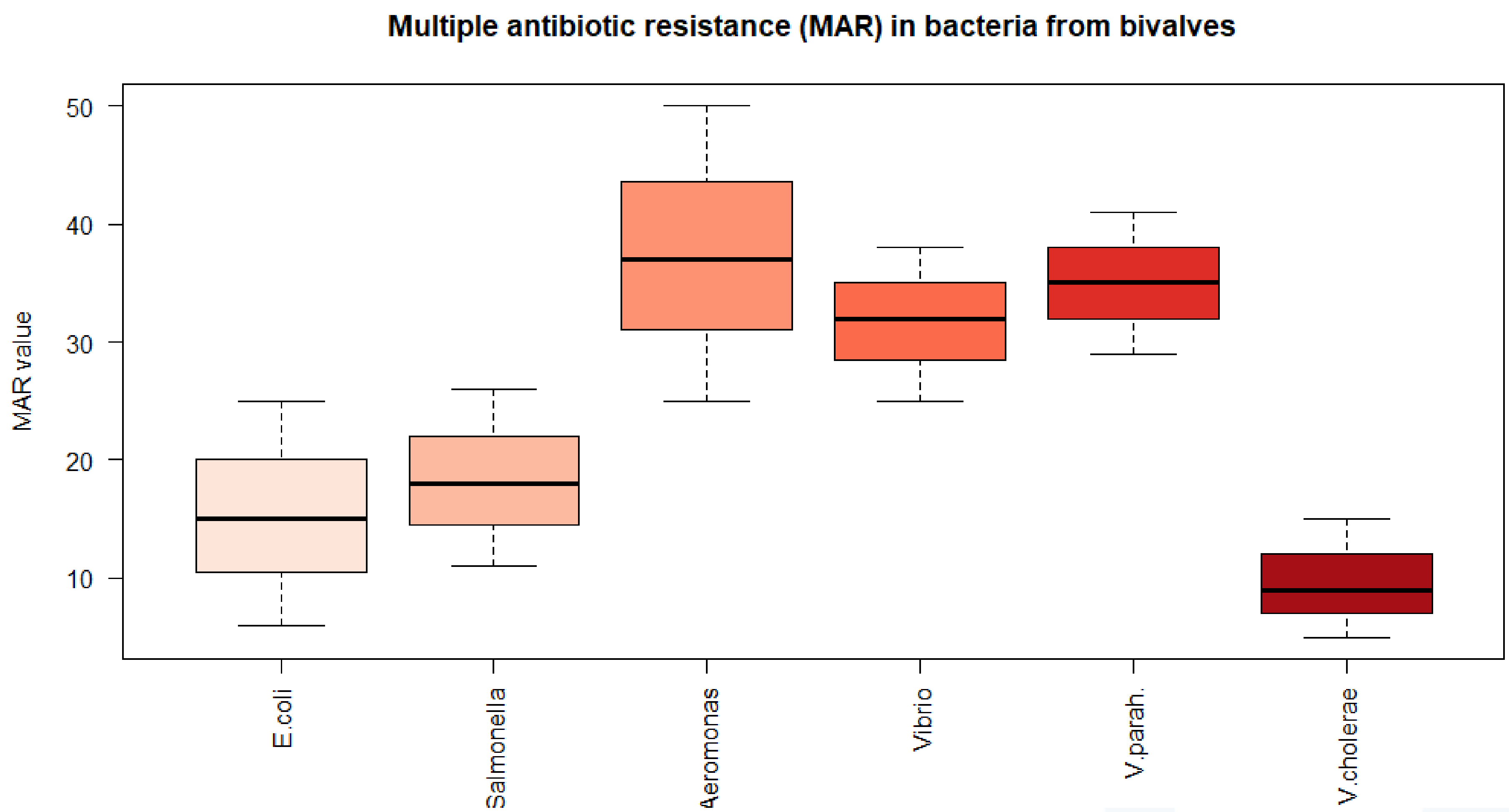
What is the prevalence of antibiotic resistance in bacteria from bivalves?



Antibiotic resistance is generally higher in marine than in terrestrial bacteria

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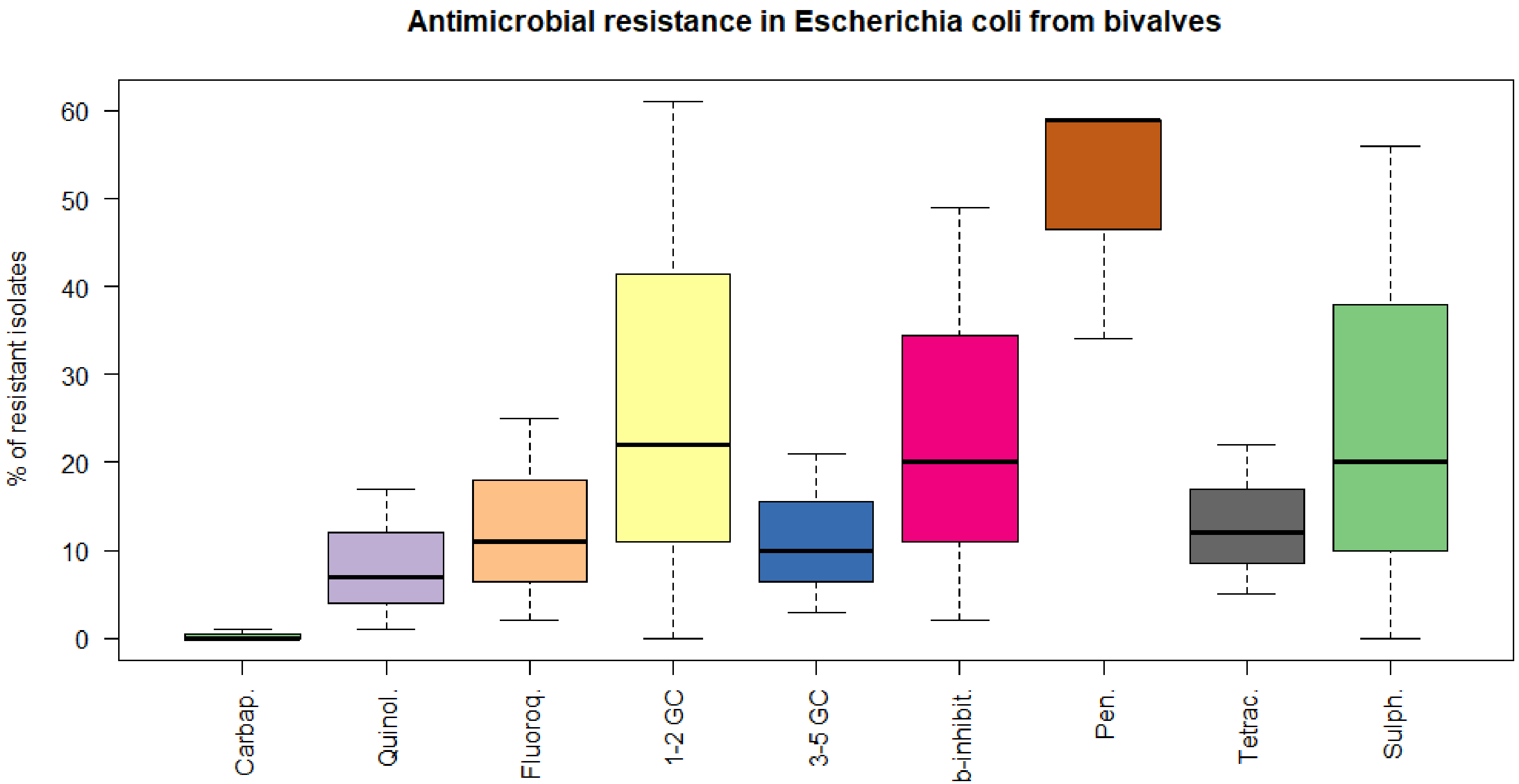
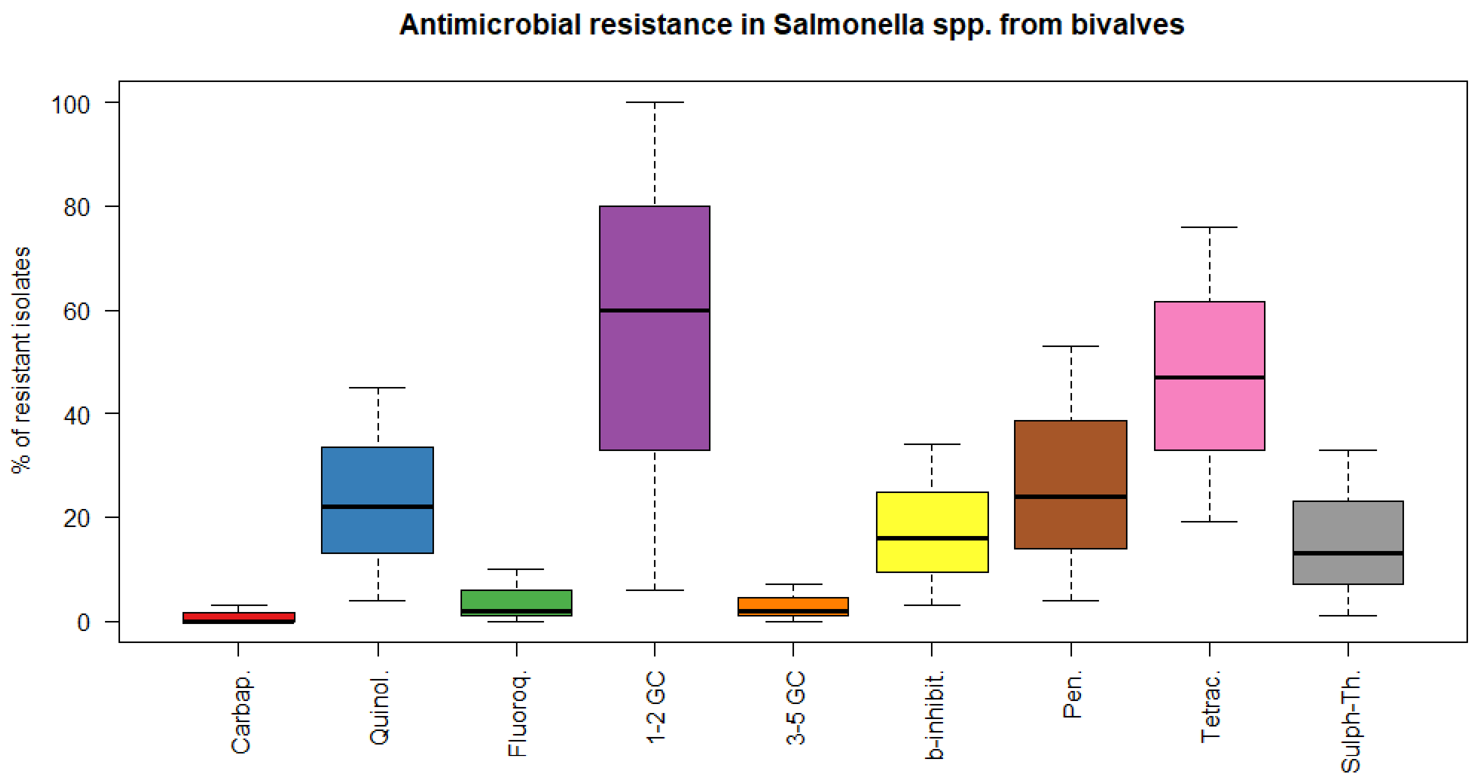
Does antibiotic resistance differ in different bacterial species or genera isolated from bivalves?



High prevalence of resistance to: sulphonamides, and beta-lactams, including penicillins, 1-2G of cephalosporins and beta lactam-beta lactamase inhibitors . Moderate levels of resistance to sulphonamides, fluoroquinolones and tetracyclines.

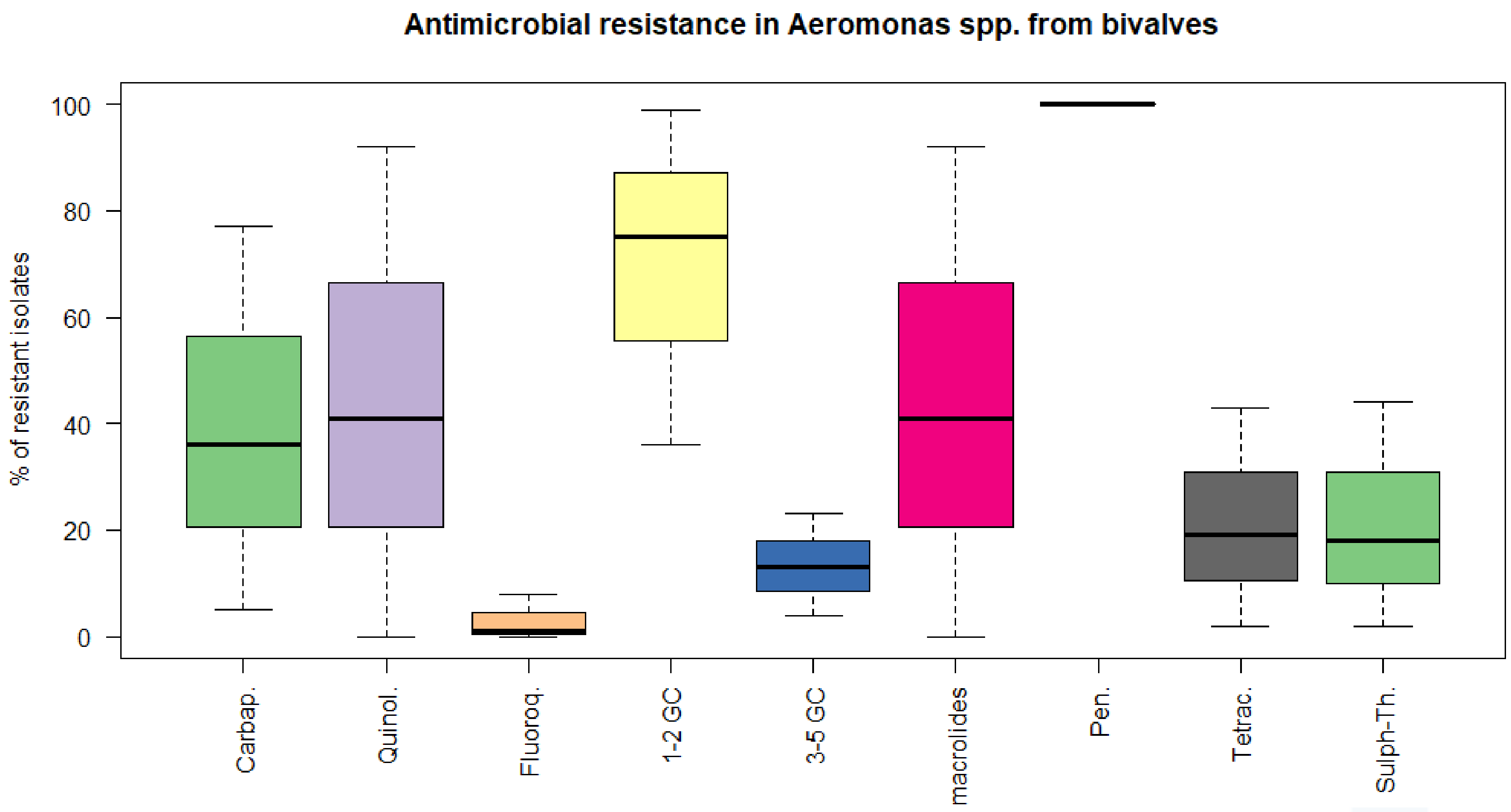
Resistance to 3-5 G of cephalosporins and fluoroquinolones, two highest priority, critically important antimicrobials (HPCIA) in approximately 10% of the isolates.

These findings suggest that bivalves carry *E.coli* resistance and, worryingly, resistant to HPCIA to humans along the food chain, posing a health risk for the health of the consumers.



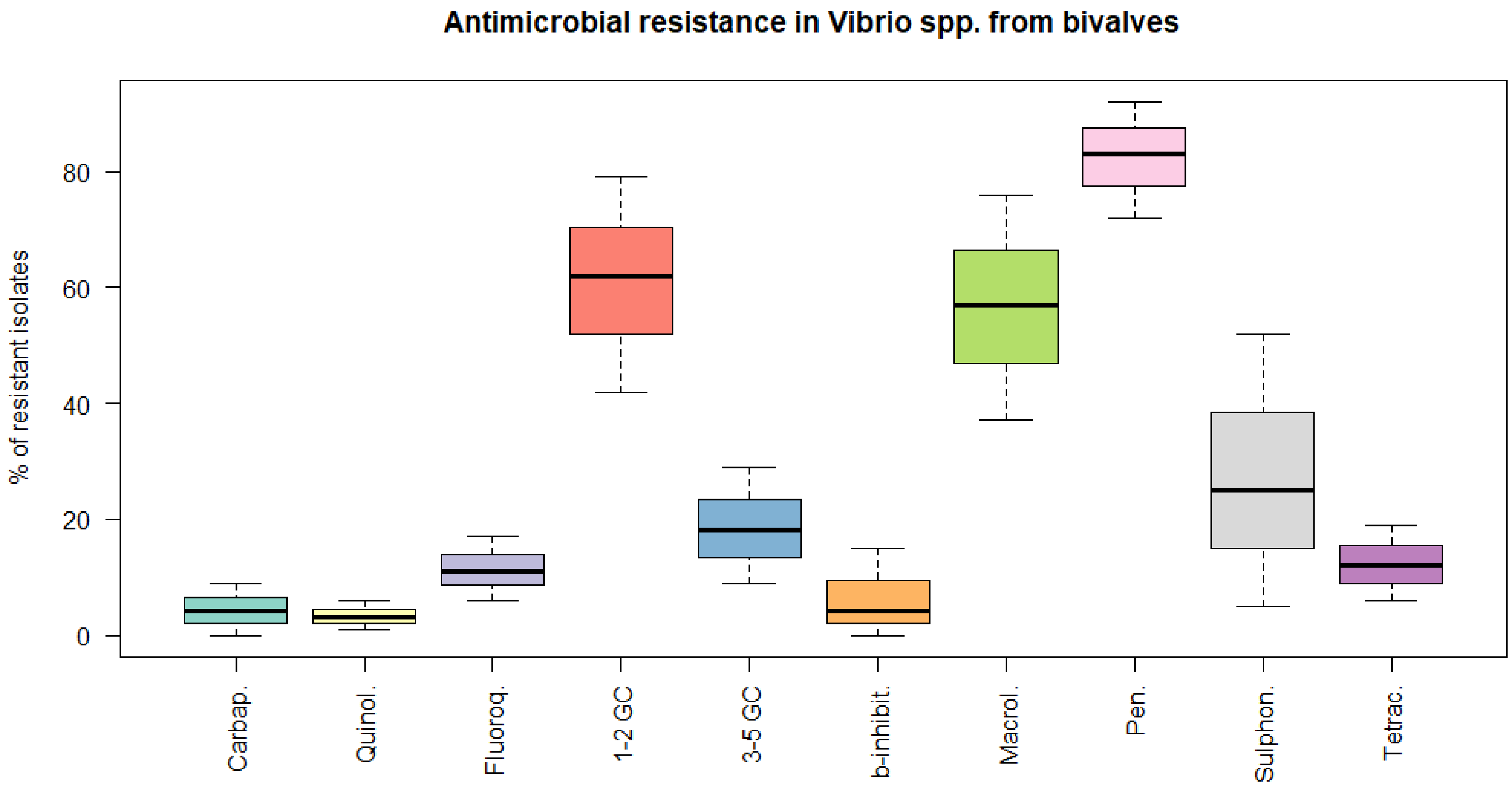
High prevalence of resistance to quinolones, 1-2G cephalosporins, tetracyclines, penicillins
Moderate levels of resistance to beta lactams-beta lactamase inhibitors and trimethoprim-sulfonamide combinations. By contrast, resistance to fluoroquinolones and third/fourth/fifth generation cephalosporins was low and resistance to carbapenems was very rare.

It should be noted that antibiotic resistance in *Salmonella* spp. varies according to the serotype to which it belongs. The prevalence of antibiotic resistance in this bacterial species in a population is strongly dependent on the dissemination of successful clones (EFSA, 2021).



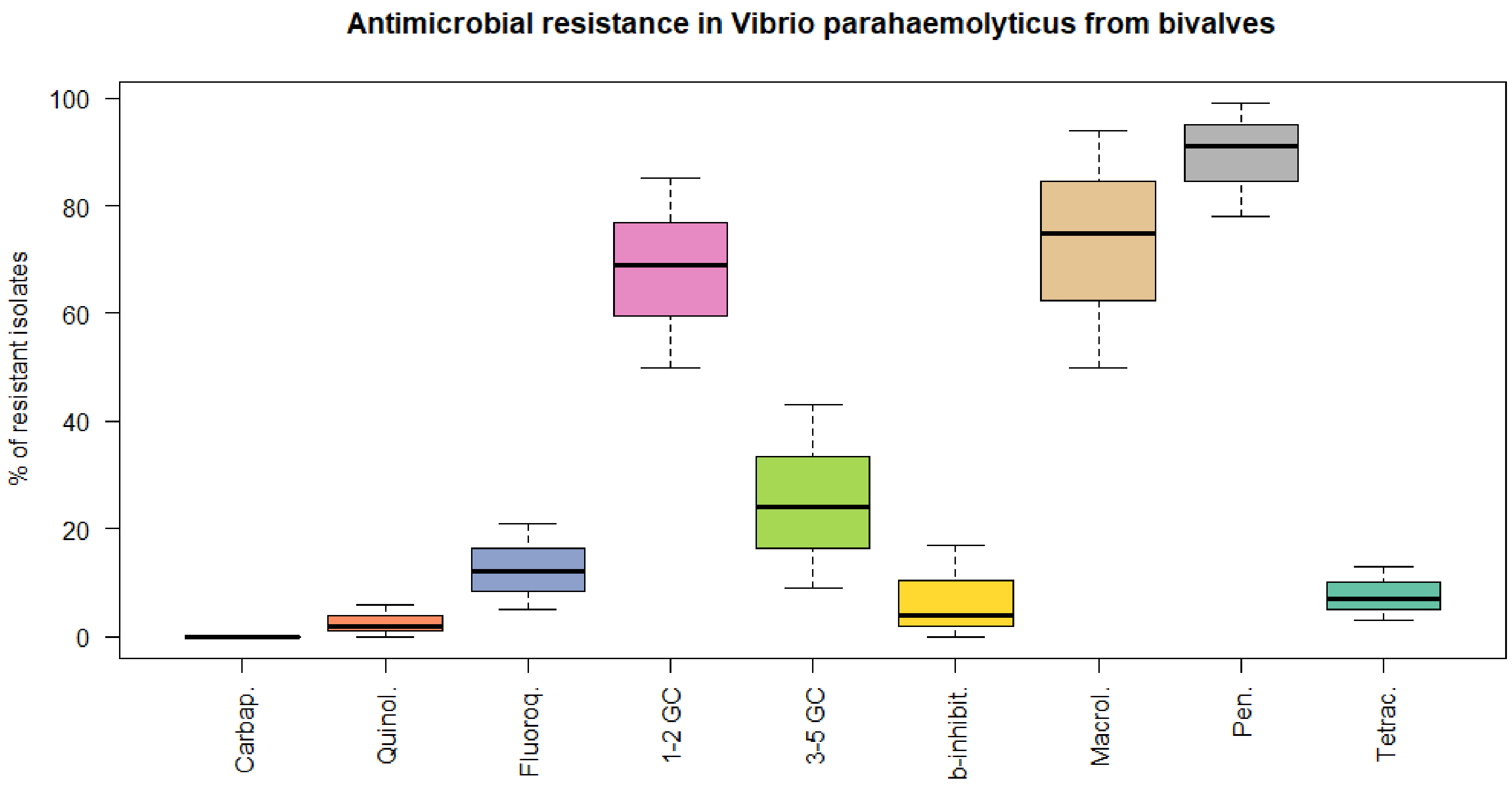
All *Aeromonas* isolates were resistant to penicillin. This finding is following what was reported by other authors, who indicated a natural resistance to penicillins and first/second generation of cephalosporins1-2 GC of this genus

Resistance to 3-5GC was detected in 13% of *Aeromonas* isolates. In this genus, beta lactamases are often encoded by plasmids, posing a threat for the possible transfer to other bacterial species via horizontal gene transfer (Piotrowska, 2017).



In Vibrio spp., a high prevalence of AMR was found to beta-lactams (penicillin, first/second generation of cephalosporins), macrolides and sulfonamides.

Resistance to penicillins is probably linked to the presence of a class A carbenicillin-hydrolyzing β-lactamase (CARB)



Elevated levels of resistance were also found for *V. parahaemolyticus*, a species associated with vibriosis in humans. Resistance to antibiotics may cause a treatment failure in case of human infection.

Our study confirms the susceptibility of *Vibrio* spp. and *V. parahaemolyticus* to tetracyclines and quinolones, two antibiotic classes recommended for the treatment of vibriosis

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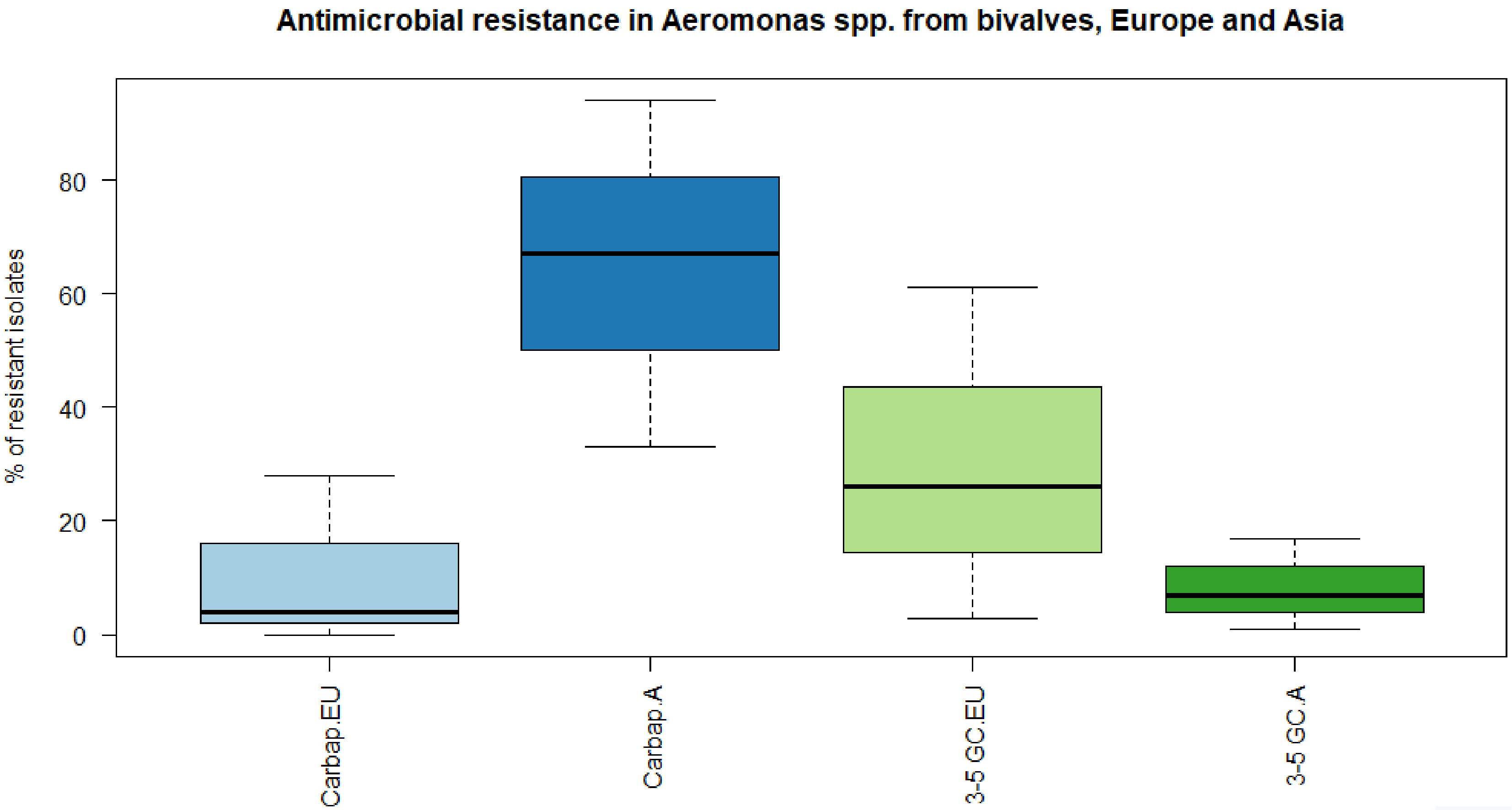
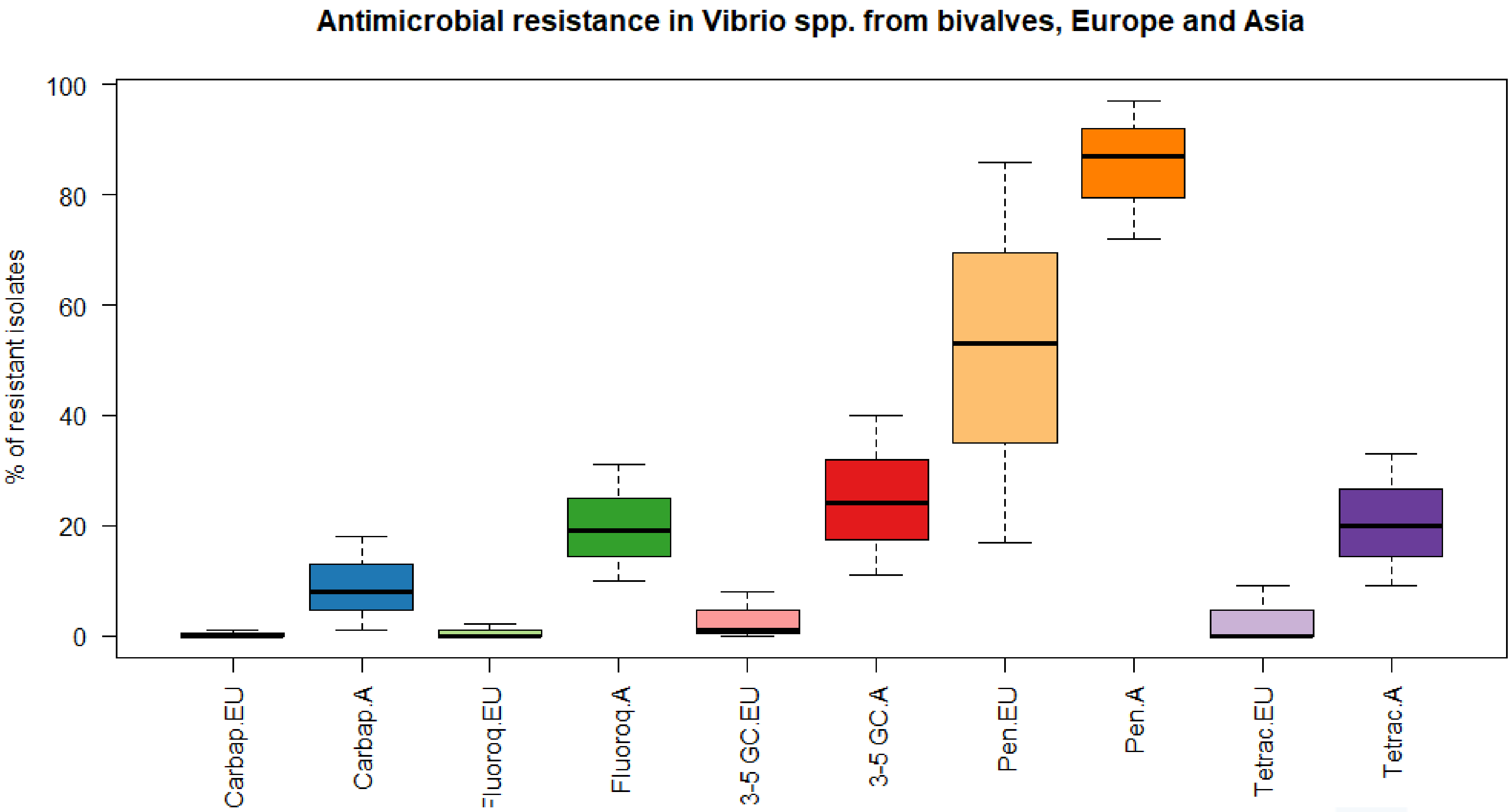
Does AMR vary in bacteria of different geographical origin?



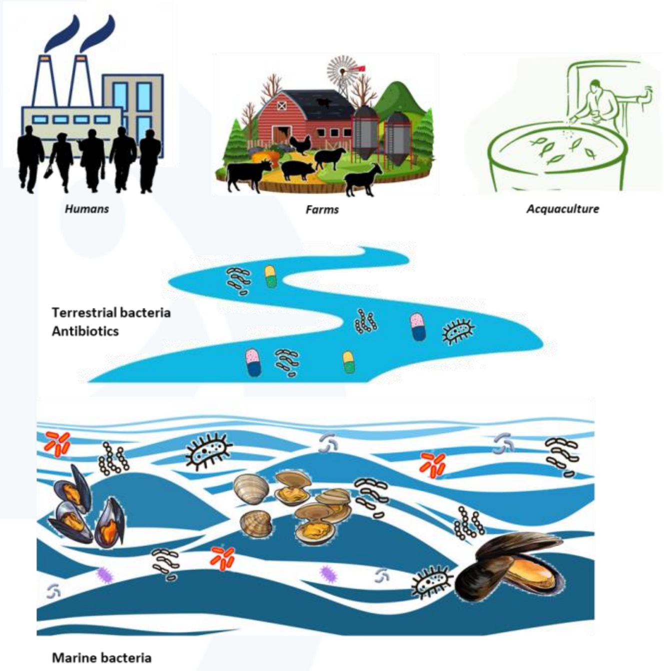
Aeromonas spp. isolates from Asia and Europe differed:
a higher prevalence of Asian isolates was resistant to carbapenems



We observed a higher prevalence of resistance to tetracyclines in *Vibrio* spp. isolated from Asia than from Europe.
The *Vibrio* spp. isolated from Asia showed higher prevalence to carbapenems, fluoroquinolones, third/fourth/fifth generation cephalosporins as compared to European isolates

Take home message

- Bivalves carry bacteria resistant to several antibiotic classes, including HPCIA
- Resistance is generally higher in marine bacteria than in bacteria from the terrestrial environment
- Resistance varies according to the geographical origin of the bivalves
- High variability of methods applied for antibiotic susceptibility testing



ASK Project –Final Report "AMR in seafood as common ground for knowledge exchange and risk assessment" EFSA, 2018

(GA/EFSA/ AFSCO/2017/01 – GA06)

Seafood differ from other major food production systems, since no standardized AMR surveillance exists, despite several studies showing that such commodities can be contaminated by various antimicrobial resistant bacteria, including both human and zoonotic pathogens

Table 1. Pre-requisites for sampling design

	Description	What we want to measure	The sample should be representative of?	Samples should be taken at	Target bacteria
Aim 1a	Estimate the risk for the consumer	Foodborne exposure of the consumer	Sampling should be calculated to be representative of consumers (e.g. human population)	Retail	*Salmonella* and *Campylobacter* *Arcobacter*, *Shigella*, *Vibrio*, *Yersinia enterocolitica*, *Plesiomonas shigelloides* and *Listeria*
Aim 1b	AMR contamination of the marine environment	Environmental exposure of people	Sampling should be representative of the production sites	Primary production	*Vibrio spp. and Aeromonas spp. Escherichia coli*
Aim 2	AMR in farmed fish	AMR in relation to AMU	Sampling should be representative of the farmed fish population	Primary production	*Vibrio spp. and Aeromonas spp.*
Aim 3	AMR in fish pathogens	Susceptibility of fish pathogens to AM	Sampling should be representative of the diseases linked to antibiotic consumption	Laboratory submissions	*Fish pathogens*

Indicator of AMR in seafood

Isolated from different types of seafood

Associated to the terrestrial or the marine environment

Resistant to freezing

Standardized culture methods available

Standardized methods for antibiotic susceptibility testing available

Antibiotic resistance in seafood

A project funded by the Italian Ministry of Health (RC004/19)

Prevalence of candidate **indicators** in different categories of seafood

- Terrestrial and marine bacteria
- Wild-caught vs. farmed-raised
- Fresh vs. frozen

AMR in bacteria from seafood
- Prevalence of resistance
- Presence of antibiotic-resistance genes (HPCIA)

Sampling

Group		N
1	Bass and brim	65
2	Anchovy	38
3	Cephalopods	68
4	Cod	66
5	Salmon	60
6	Bivalves	61
7	Crustaceans	64

Over one year

Two regions
Supermarket

Fresh and frozen

Data on:
Wild caught vs farmed
FAO zone of origin

E. coli

E. coli ESBL AmpC

E. coli R-Carbap.

Enterococcus spp.

Aeromonas spp.

Vibrio spp.

Enterococcus spp.

Seafood category samples	Negative samples		Positive samples		Total
	N	%	N	%	
1 (bass)	30	46%	35	54%	65
2 (anchovy)	19	50%	19	50%	38
3 (cephalopods)	17	25%	51	75%	68
4 (cod)	26	39%	40	61%	66
5 (salmon)	14	23%	46	77%	60
6 (bivalves)	14	23%	47	77%	61
7 (crustaceans)	14	22%	50	78%	64
Total	134	32%	288	68%	422

in all samples prevalence exceed 50%

Overall prevalence: 68,25% (IC95%: 63,66%-72,51%).

Enterococcus spp.

Category	Odds ratio (OR)	CI 95%	p-value
1 (bass)	1	-	-
2 (anchovy)	0,86	0,36-2,06	0,7061
3 (cephalopods)	2,57	1,16-5,75	0,0107
4 (cod)	1,32	0,62-2,80	0,4342
5 (salmon)	2,82	1,22-6,62	0,0076
6 (bivalves)	2,88	1,25-6,75	0,0063
7 (crustaceans)	3,06	1,34-7,16	0,0036
frozen vs fresh	1.69	1.08-2.70	0.0156
wild-caught vs farm-raised	1.1	0.71-1.70	0.6540

Enterococcus spp.

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Frozen seafood had increased odds of contamination

		E. faecalis	*E. faecium*	*E. casseliflavus*	*E. hirae*	*E. thailandicus*	**Others***	**Total**
Chloramphenicol	R	5 (8.77)	1 (5)	0	0	1 (20)	0	7 (6.86)
	I	8 (14.03)	2 (10)	0	0	0	0	10 (9.80)
Ciprofloxacin	R	4 (7.01)	11 (55)	3 (25)	1 (25)	1 (20)	0	20 (19.60)
	I	42 (41.17)	7 (35)	7 (58.33)	1 (25)	3 (60)	1 (25)	61 (59.80)
Erythromycin	R	9 (15.79)	3 (15)	0	2 (50)	2 (40)	0	16 (15.68)
	I	16 (28.07)	13 (65)	10 (83.33)	0	1 (20)	0	40 (39.21)
Linezolid	R	1 (1.75)	1 (5)	0	0	0	0	2 (1.96)
	I	6 (10.52)	3 (15)	2 (16.66)	1 (25)	2 (40)	0	14 (13.72)
Quinupristin/	R	49 (85.96)	2 (10)	3 (25)	0	0	0	54 (52.94)
Dalfopristin	I	3 (5.26)	6 (30)	9 (75)	2 (50)	5 (100)	2 (50)	27 (26.47)
Streptomycin	R	0	2 (10)	0	1 (25)	0	0	3 (2.94)
Tetracycline	R	14 (24.56)	7 (35)	0	2 (50)	3 (60)	2 (50)	28 (27.45)
	I	1 (1.75)	0	0	0	0	0	1 (0.98)
Vancomycin	R	0	0	0	0	0	0	0
	I	0	0	0	0	0	1 (25)	1 (0.98)
Nitrofurantoin	R	0	0	0	0	0	0	0
	I	0	17 (85)	0	4 (100)	5 (100)	1 (25)	27 (26.47)
Penicillin	R	0	0	1 (8.33)	0	1 (20)	0	2 (1.96)
Multi-resistant		43 (75.4)	12 (60)	10 (83.3)	1 (25)	4 (80)	1 (25)	71 (69.6)
Full susceptible		0	1 (10)	0	0	0	2	3 (2.94)
Total		57	20	12	4	5	4	102

		E. faecalis	*E. faecium*	*E. casseliflavus*	*E. hirae*	*E. thailandicus*	Others*	Total
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Antibiotic resistance in seafood

A project funded by the Italian Ministry of Health (RC004/19)


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Prevalence of Antimicrobial Resistance in Select Bacteria From Retail Seafood—United States, 2019

Heather Tate[1], Sherry Ayers[1], Epiphanie Nyirabahizi[1], Cong Li[1], Stacey Borenstein[1], Shenia Young[1], Crystal Rice-Trujillo[1], Sanchez Saint Fleurant[1], Sonya Bodeis-Jones[1], Xunde Li[2], Melissa Tobin-D'Angelo[3], Victoriya Volkova[4], Rachel Hardy[5], Lisa Mingle[6], Nkuchia M. M'ikanatha[7], Laura Ruesch[8], Chris A. Whitehouse[1], Gregory H. Tyson[1], Errol Strain[1] and Patrick F. McDermott[1]*

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Xian-Zhi Li,
Health Canada, Canada

- The **wild-caught** category resulted in an increased OR for the presence of a multi-resistant isolate as compared to belonging to the farm-raised category (OR: 2.89, CI95%:1.21-6.89, p=0.017).

- Furthermore, categories 3, **cephalopods**, (OR: 19.5, CI95%:1.99-190.88, p=0.011) and 5, **salmon** (OR: 9.75; CI95%:1.59-59.69; p=0.014), were associated with higher ORs for the presence of a multi-resistant isolate than category 1 (bass and brim)

Enterococcus as an indicator of AMR in seafood

- *Enterococcus* spp. is common in all the categories of seafood, in wild-caught and farmed seafood and resist freezing
- Resistance to antibiotics is common and for some antibiotics, varies among different species
- *Enterococcus* isolated from seafood may be resistant to HPCIA

In conclusion

- Seafood is frequently contaminated by AR bacteria, including bacteria resistant to HPCIA
- Resistance may be transferred to humans via the foodborne route, workers exposure or the environment
- Seafood is highly globalized and it is often consumed raw
- For AMR in seafood, few surveillance plans are currently in place worldwide
- Surveillance should target marine and terrestrial bacteria
- Reduction of antibiotic use still remains the main mitigation measure

Thank you
for your attention!