



Antimicrobial resistance in food

Pier Sandro Cocconcelli

Università Cattolica del Sacro Cuore

Italy

pier.cocconcelli@unicatt.it



AMR in the food chain

1. The data on AMR in the food chain

The EU reports

2. Risk assessment of AMR in food

RTE Foods:
Hazards characterisation
and Exposure assessment

3. The measures for risk mitigation

The EFSA approach



UNIVERSITÀ
CATTOLICA
del Sacro Cuore



THE NEW EU ONE HEALTH ACTION PLAN AGAINST ANTIMICROBIAL RESISTANCE



1. MAKING THE EU A BEST PRACTICE REGION



2. BOOSTING RESEARCH, DEVELOPMENT AND INNOVATION ON AMR



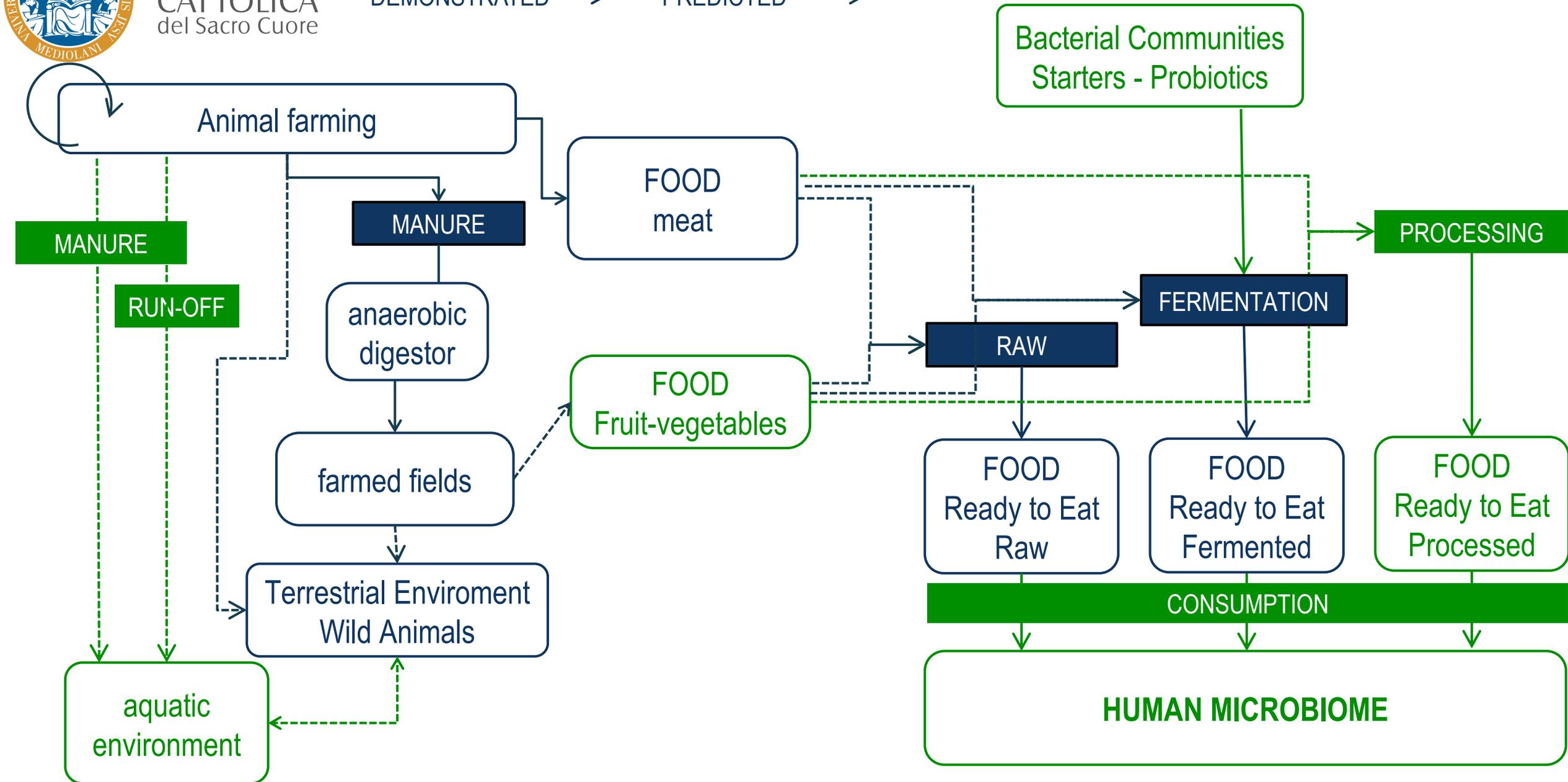
3. SHAPING THE GLOBAL AGENDA



IS AMR A FOOD RISK?



DEMONSTRATED → PREDICTED →





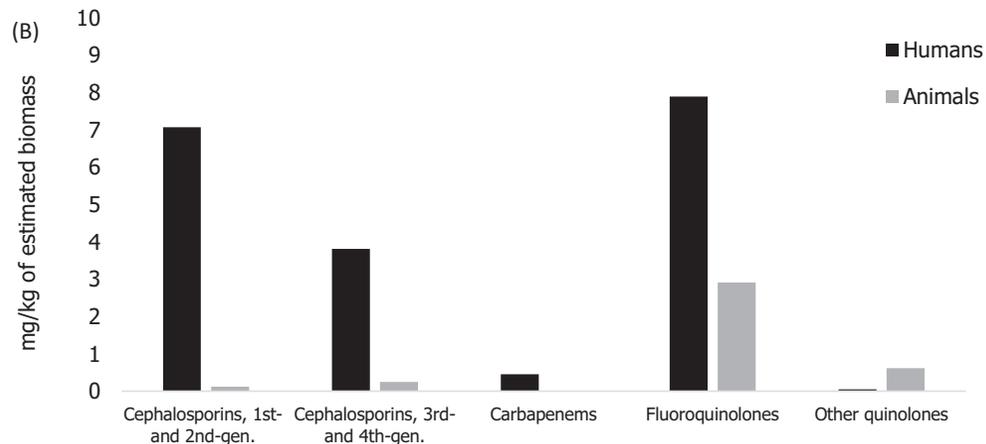
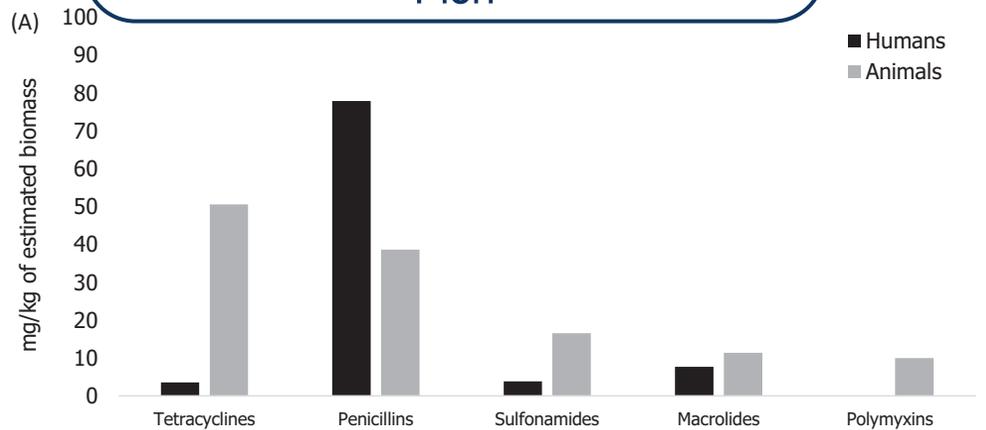
SCIENTIFIC REPORT

APPROVED: 28 June 2017

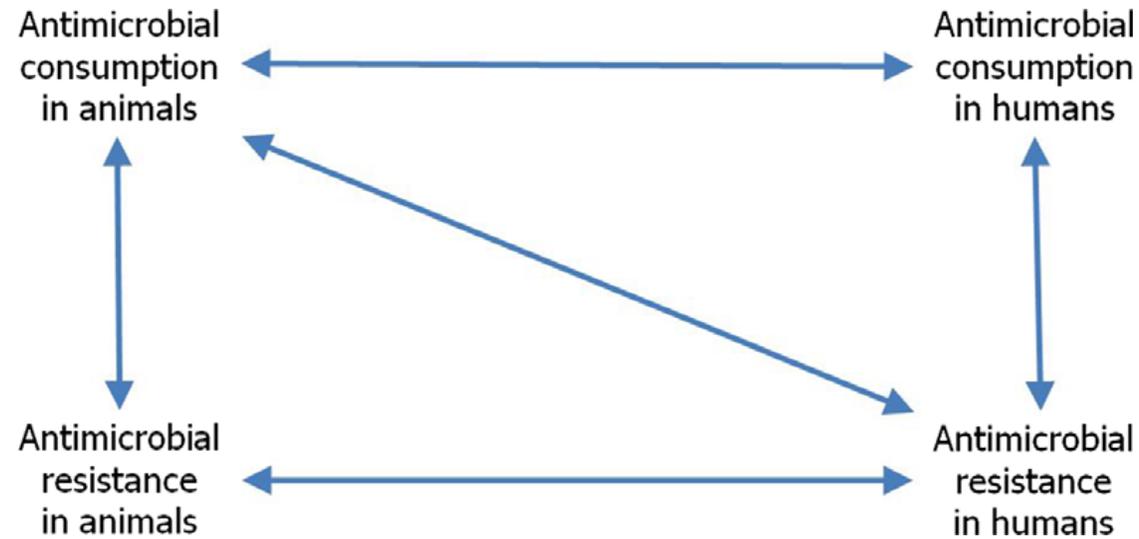
doi: 10.2903/j.efsa.2017.4872

ECDC/EFSA/EMA second joint report on the integrated analysis of the consumption of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from humans and food-producing animals

Food Producing Animals
Cattle - Pigs
Avian species
Fish



Consumption of antimicrobials (tonnes of active substance)		
Humans	Animals	Total
3,821	8,927	12,720

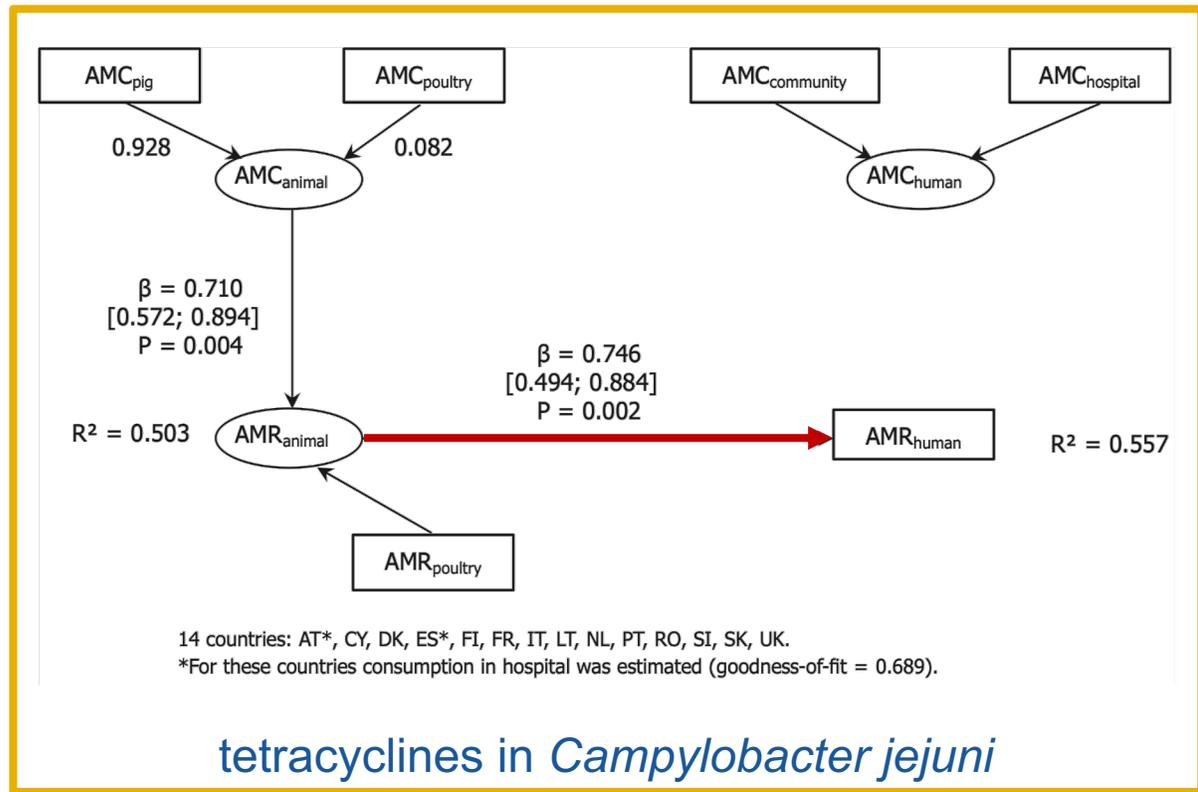
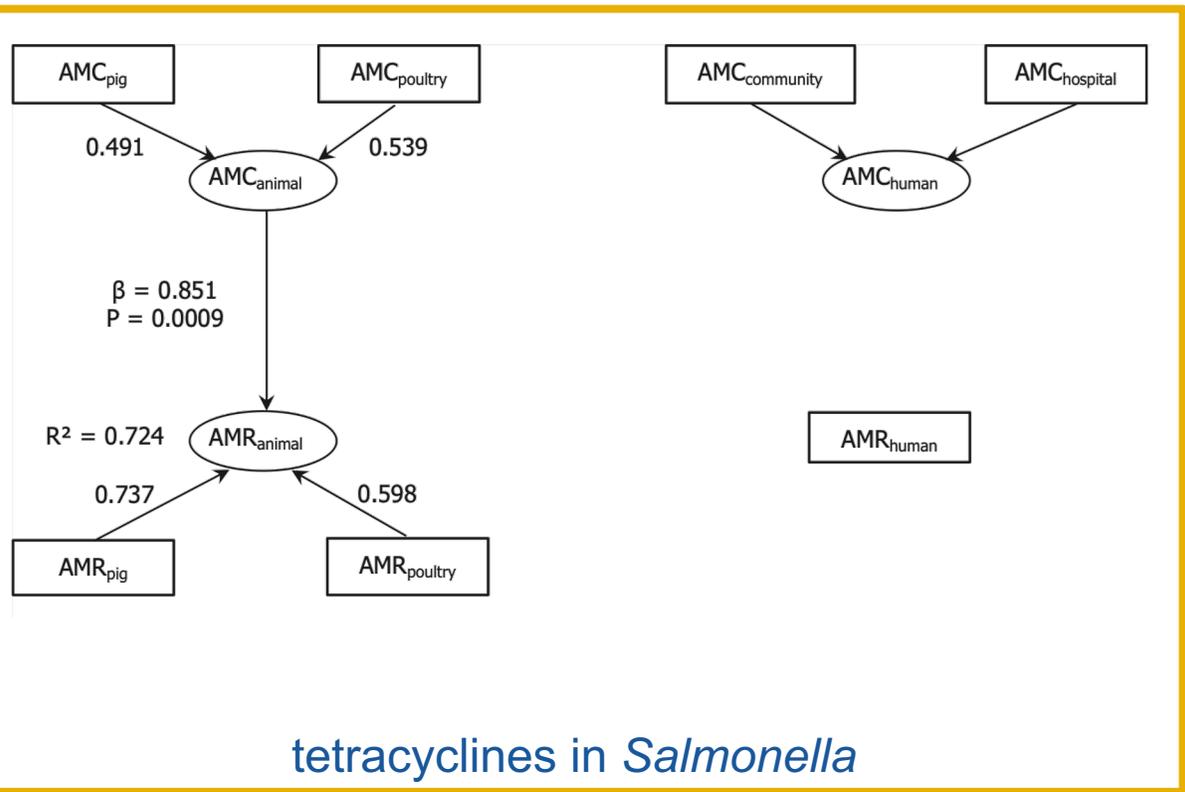


Note: The relationship between AMC in humans and AMR in food-producing animals was not addressed in this report.

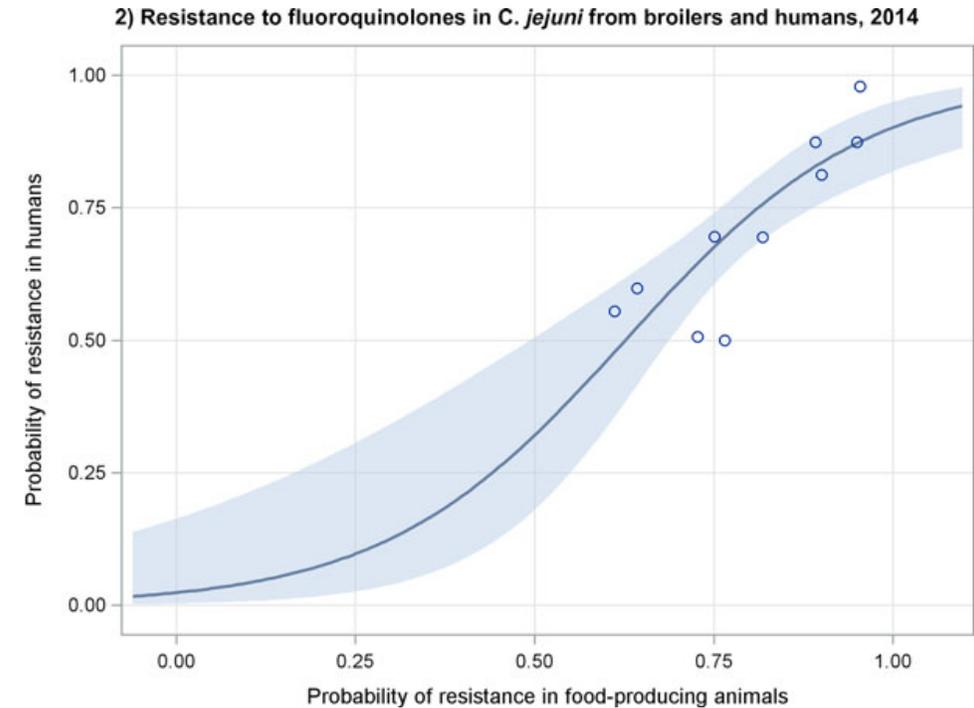
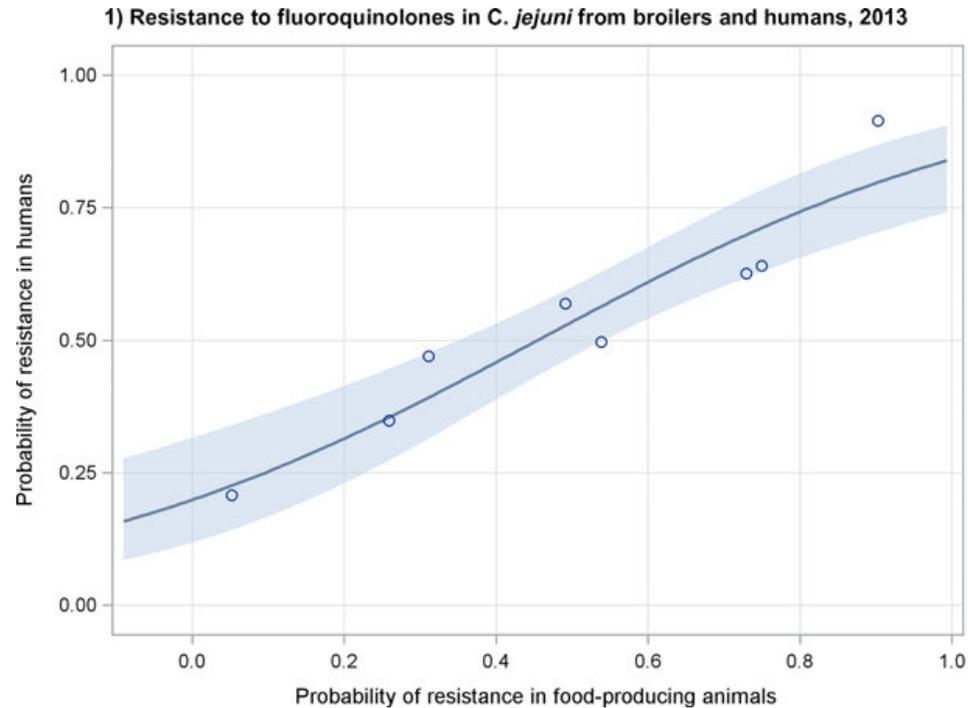
Figure 1: Available sets of data related to AMC and AMR in humans and food-producing animals in the reporting countries and the possible relationships investigated in this report

- AMC in humans
- AMR in humans
- AMC in food-producing animals
- AMR in food-producing animals

Partial Least Squares Path Modeling (PLS-PM).
potential relationships between antimicrobial resistance in bacteria from humans (AMR_{human}) and antimicrobial consumption in humans (AMC_{human}), antimicrobial consumption in animals (AMC_{animal}) (whether as direct or indirect influential factor), and antimicrobial resistance in bacteria in animals (AMR_{animal})



Logistic regression analysis curves of the AMR consumption in food producing animals and the probability of resistance



Dots represent countries included in the analysis.

Figure 24: Logistic regression analysis curves of the probability of resistance to fluoroquinolones in *Campylobacter jejuni* from food-producing animals and humans, (1) 2013 and (2) 2014 (see also Table 21)



AMR in the food chain

1. The data on AMR in the food chain

The EU reports

2. Risk assessment of AMR in food

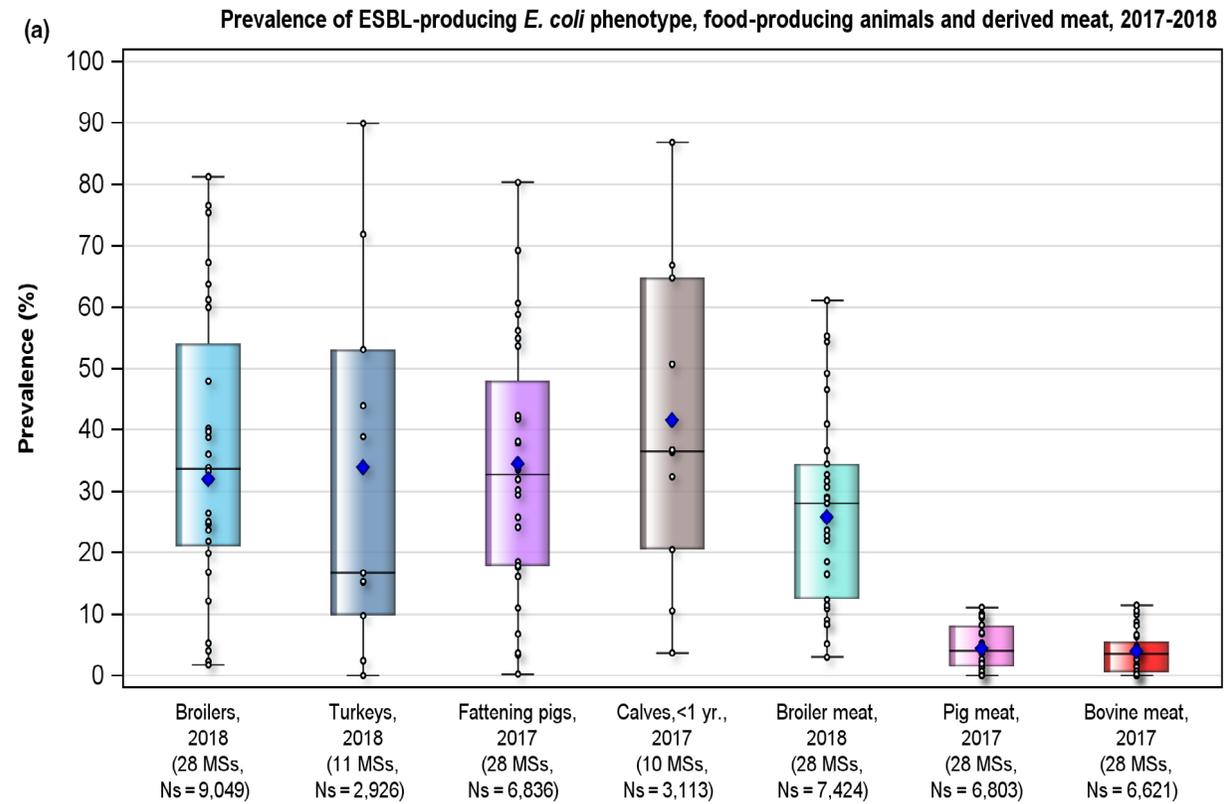
RTE Foods:
Hazards characterisation
and Exposure assessment

3. The measures for risk mitigation

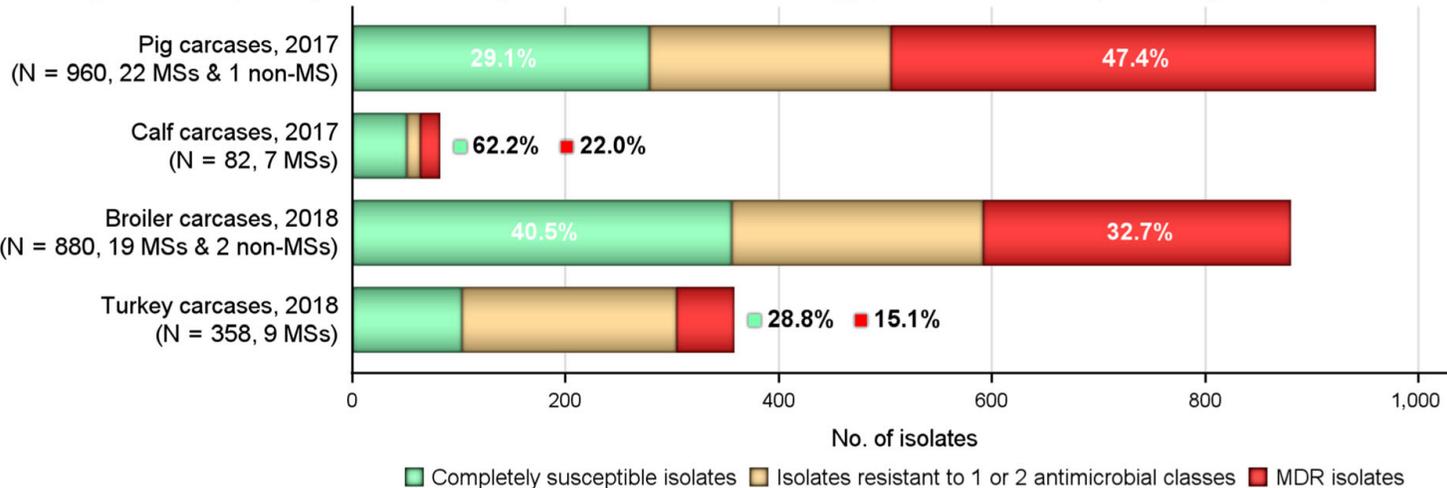
The EFSA approach

The European Union Summary Report on Antimicrobial Resistance in zoonotic and indicator bacteria from humans, animals and food in 2018/2019

European Food Safety Authority and European Centre for Disease Prevention and Control



Complete susceptibility and multi-drug resistance in *Salmonella* spp., carcasses of food-producing animals, 2017/2018

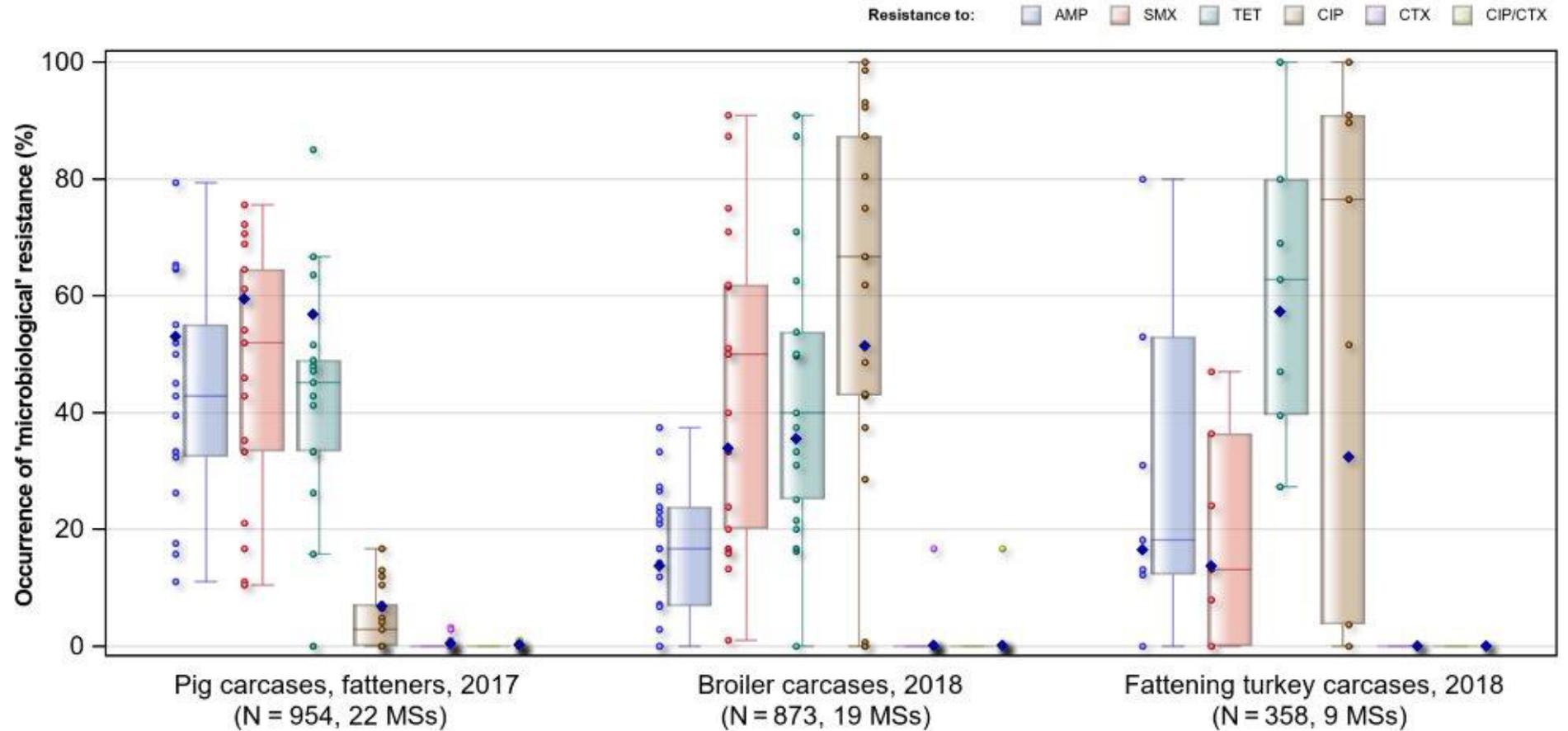


Salmonella
Campylobacter
Escherichia coli
 MR-*Staphylococcus aureus*
 live animals and carcasses/raw meat



- Differences between the systems for collection and reporting of data on AMC and AMR in bacteria from humans and food-producing animals hamper direct comparisons.
- Nevertheless, in most cases, AMC was positively associated with AMR in both animals and humans.

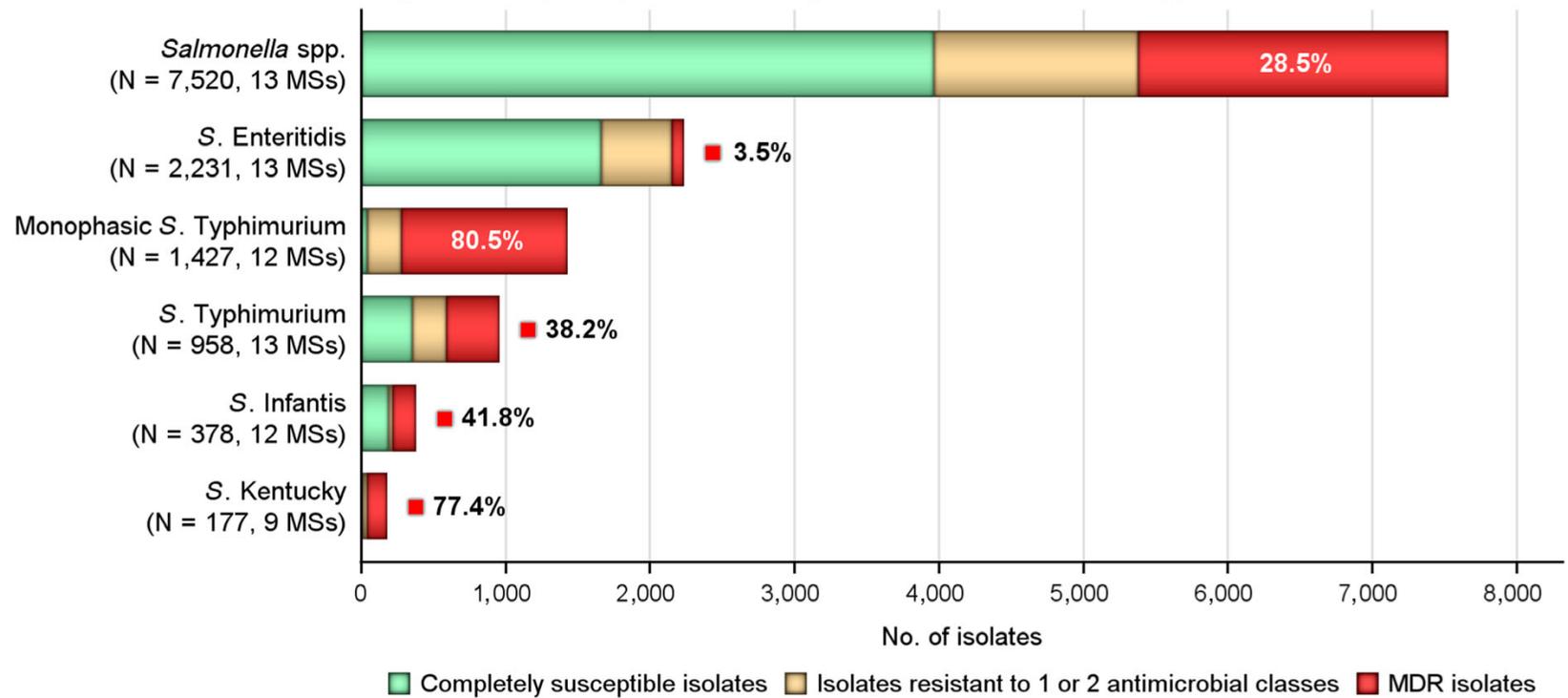
Occurrence of resistance in *Salmonella* spp., carcasses of food-producing animals, 2017-2018



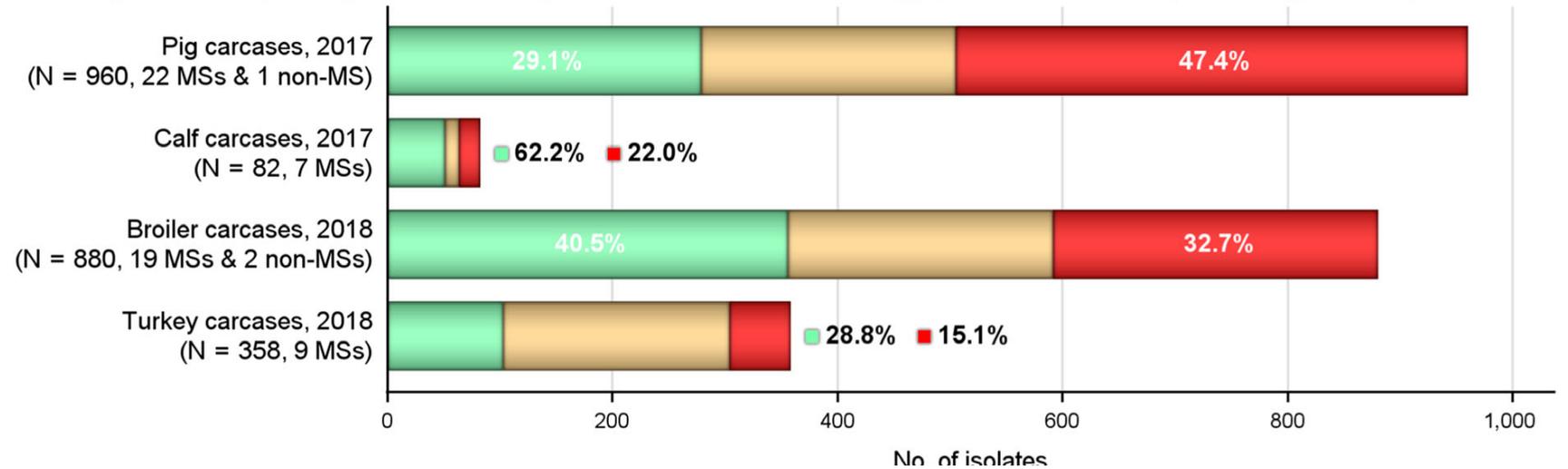
AMP: ampicillin; CIP: ciprofloxacin; CTX: cefotaxime; SMX: sulphonamides; TET: tetracyclines.



Complete susceptibility and multi-drug resistance in *Salmonella* spp., isolates from humans, 2018



Complete susceptibility and multi-drug resistance in *Salmonella* spp., carcasses of food-producing animals, 2017/2018





AMR in the food chain

1. The data on AMR in the food chain

The EU reports

2. Risk assessment of AMR in food

RTE Foods:
Hazards characterisation
and Exposure assessment

3. The measures for risk mitigation

The EFSA approach



AMR as a food risk: Gaps of Knowledge

Hazard Characterization

- The prevalence of AMR species in RTE food
- The prevalence of AMR genes in RTE food
- AMR in strains intentionally used in the food chain

Exposure

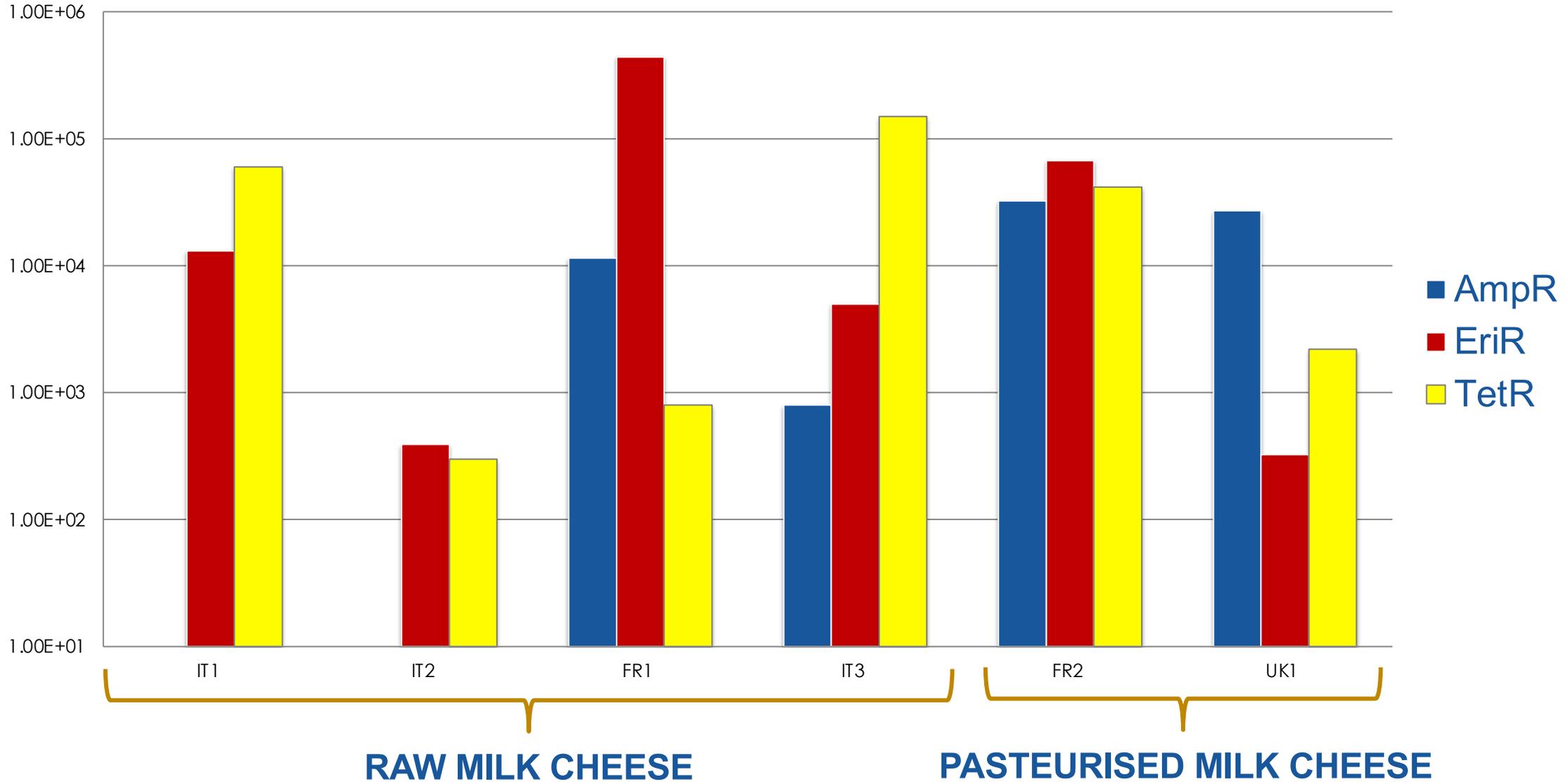
- How many AMR bacteria from RTE foods are ingested?
- What the rate of horizontal gene in food?

Risk Characterization

- What's the risk dimension?



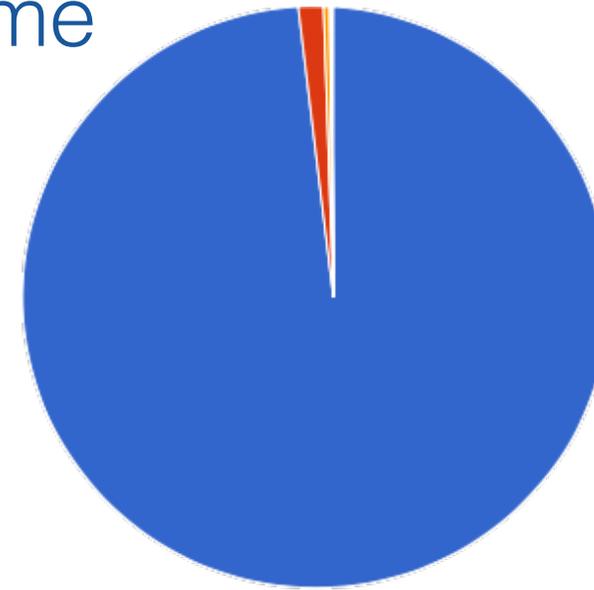
Prevalence of AMR bacteria in cheese at the consumption stage



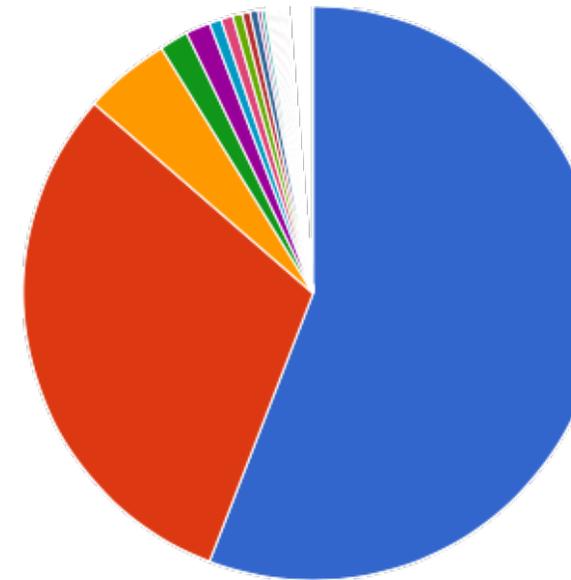
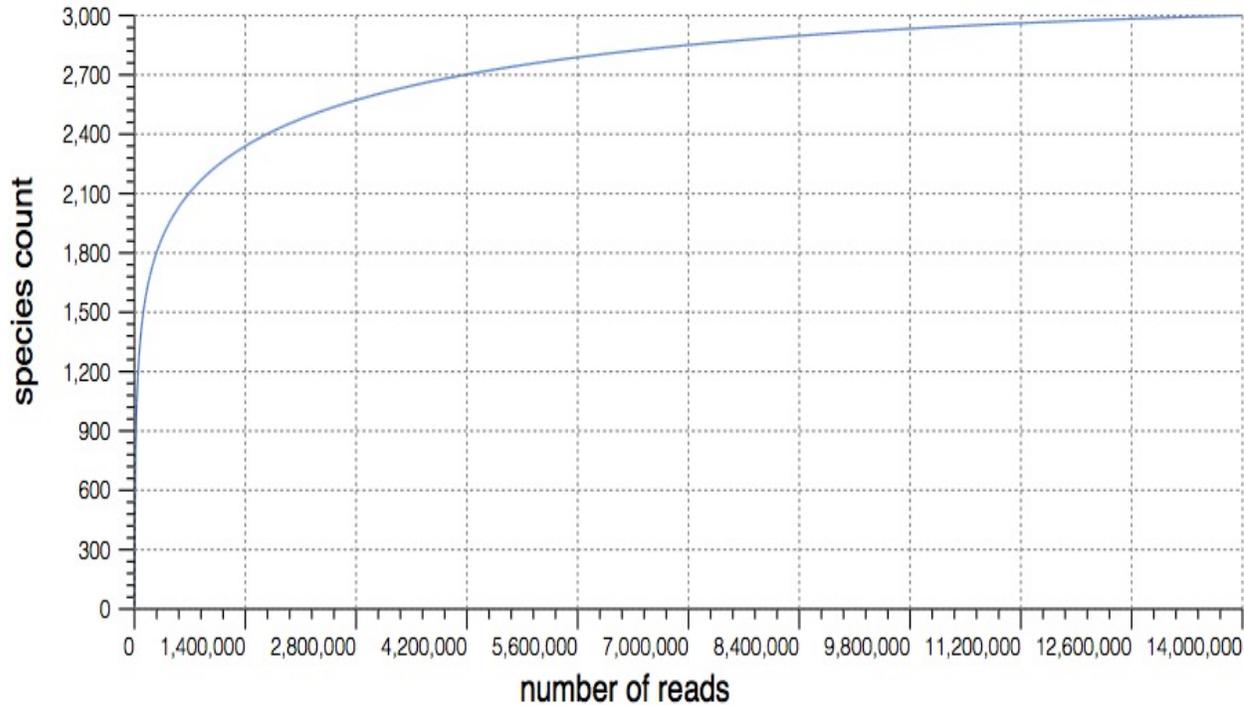


Cheese Metagenome Metaresistome

13,166,058 sequences totaling
3,724,678,492 basepairs with an
average length of 283 bps.



- Bacteria - 84,981,466 (98.18%)
- Viruses - 1,193,212 (1.38%)
- None - 218,858 (0.25%)
- other sequences - 78,580 (0.09%)
- Eukaryota - 55,309 (0.06%)
- Archaea - 25,161 (0.03%)
- unclassified sequences - 1,745 (0.00%)

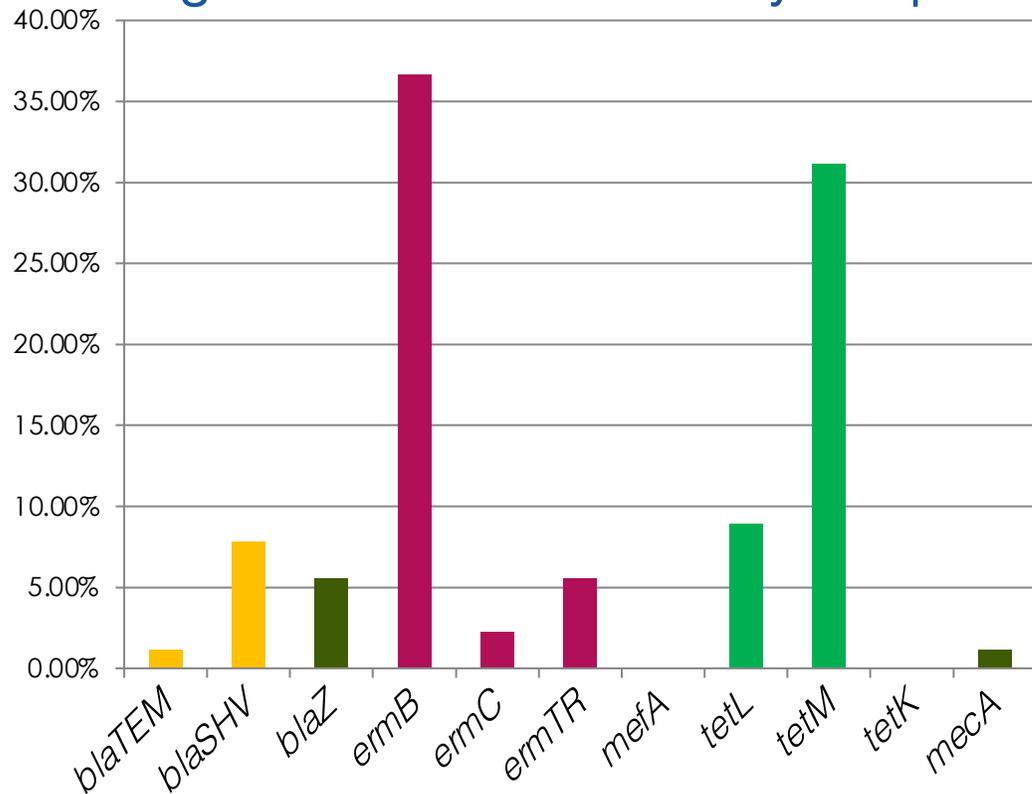


- Lactococcus - 48,329,268 (55.84%)
- Streptococcus - 26,395,216 (30.50%)
- Enterococcus - 4,245,026 (4.90%)
- Lactobacillus - 1,400,310 (1.62%)
- unclassified (derived from Siphoviridae)
- Listeria - 646,035 (0.75%)
- Staphylococcus - 556,485 (0.64%)
- Bacillus - 491,941 (0.57%)
- Leuconostoc - 365,784 (0.42%)
- Clostridium - 336,749 (0.39%)
- None - 218,865 (0.25%)
- Escherichia - 144,760 (0.17%)
- Thermus - 122,462 (0.14%)
- Yersinia - 95,536 (0.11%)



Cultivation based approach vs metagenomic (metaresistome)

AMR genes distribution in dairy samples



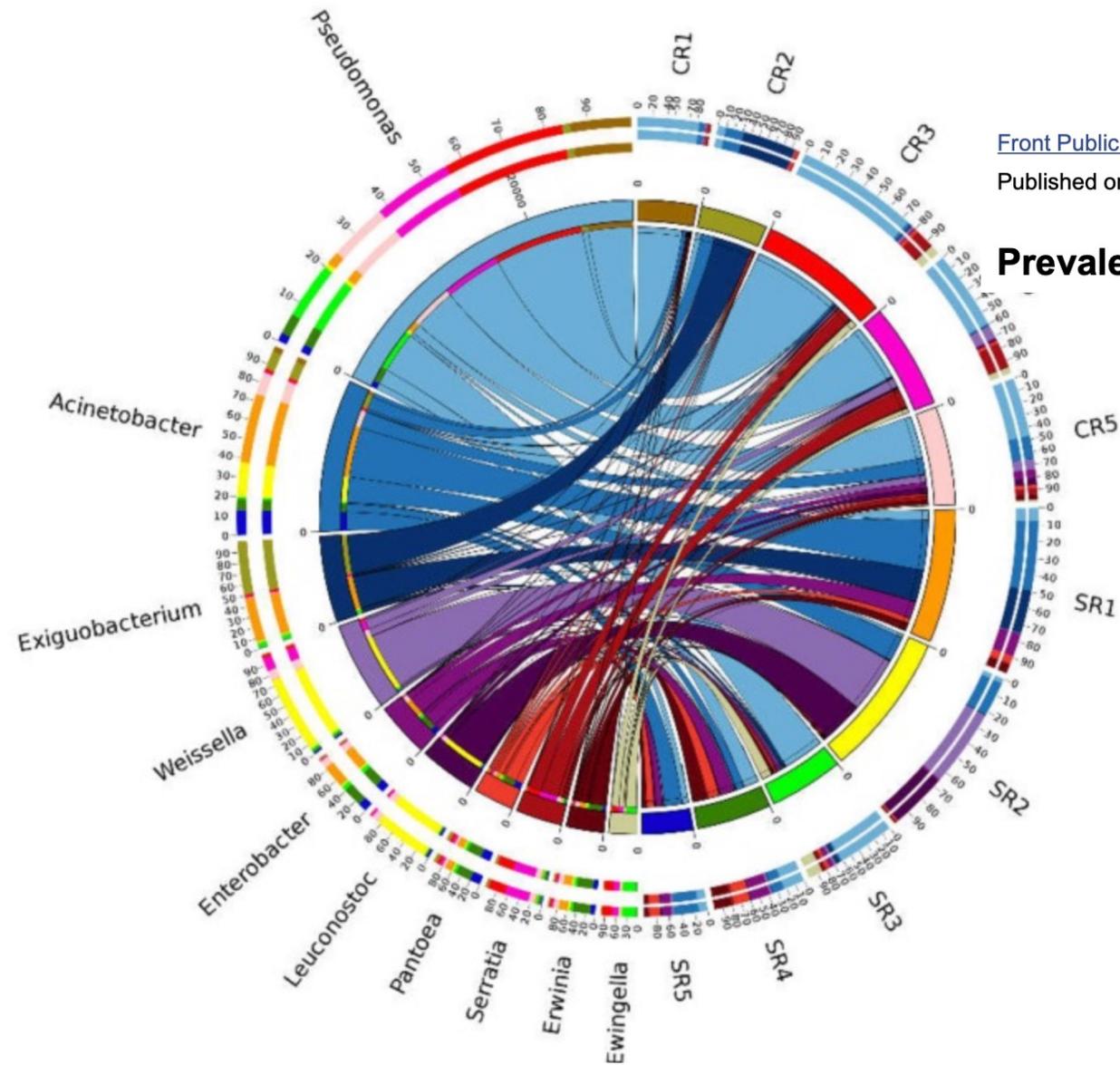
AMR: Metaresistome

ARO:3000186
 ARO:3000250
 ARO:3000375
 ARO:3000894
 ARO:3003373
 ARO:3002608
 ARO:3002578
 ARO:3003209
 ARO:3002724
 ARO:3003109

tetM
ErmC
ErmB
TEM-24
acrR
aadA8
AAC(6')-Ib7
FosA5
QnrB10
msrE

"Enterococcus faecium"
 "Staphylococcus aureus"
 "Streptococcus pneumoniae"
 "Enterobacter aerogenes"
 "multidrug resistance"
 "Pseudomonas aeruginosa"
 "Shigella flexneri"
 "Enterobacter cloacae"
 "Acinetobacter baumannii"
 "Enterobacter cloacae"

Prevalence of Antibiotic Resistome in Ready-to-Eat Salad

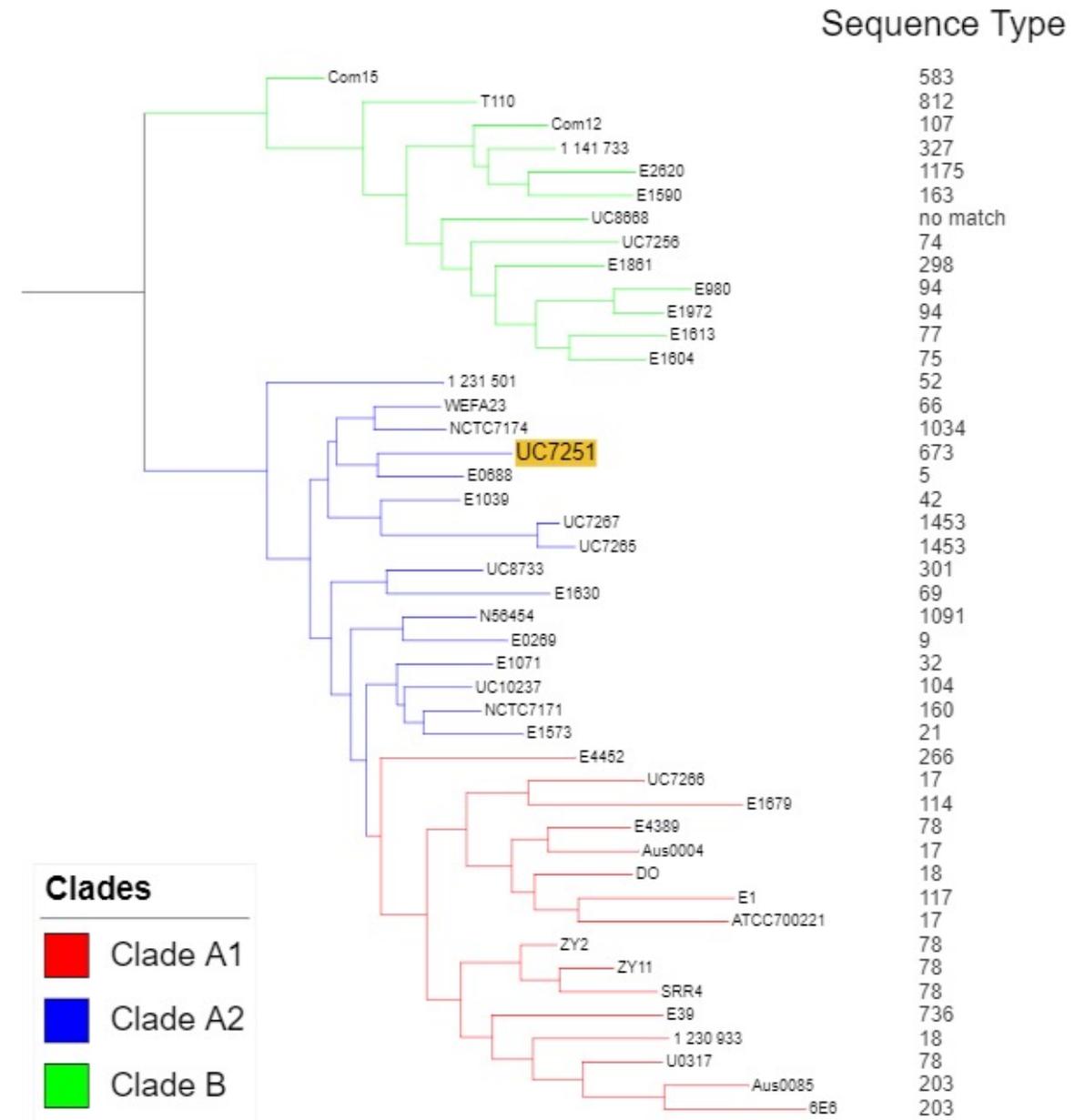


Composition of the 10 most prevalent bacterial genera detected from all samples. Data were depicted by Circos software. Length of the bars (sites) represent the percentage of the respective genus from each sample. Length of the bars (genus) represents the percentage the sample contributes to the proportion of each bacterial genus.

The multidrug resistant *E. faecium* UC7251 isolated from fermented sausage

- The population structure of *E. faecium* is divided into distinct clades, clade A containing the hospital-associated (HA) clade A and the community-associated (CA) clade B.
- A further split within clade A was identified, namely clade A2, consisting mostly of animal-associated isolates, while clinical isolates are grouped in sub-clade A1.
- This separation most likely derived from the introduction of antibiotics both in clinical and agricultural settings.
- UC7251= clade A2

ee scale: 0.1





Antimicrobial resistance: UC7251 Multi-drug resistant strain

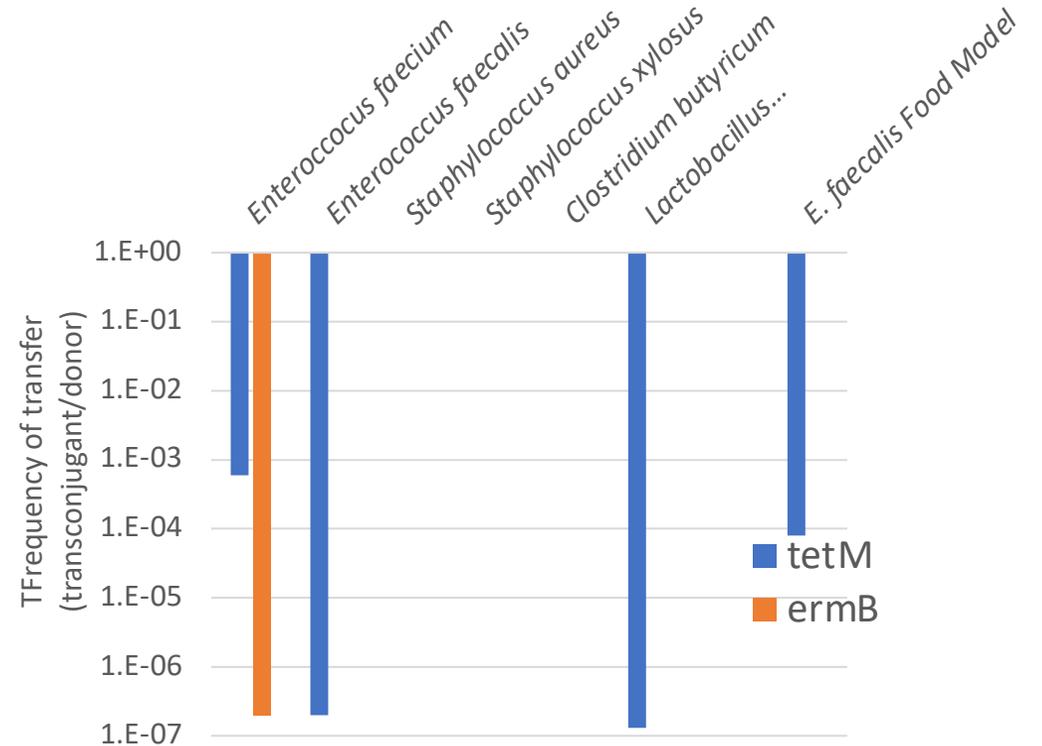
- EFSA published a scientific opinion on the safety of the use of *E. faecium* in animal nutrition, which can be considered as safe if the ampicillin MIC is ≤ 2 mg/L and, it does not carry the genetic elements IS16, *esp*, *hylEfm* (EFSA, 2012).
- UC7251:**
 - does not carry virulence factors defined by EFSA
 - Ampicillin resistance was demonstrated to be linked to the presence of a hybrid-like PBP5 (PBP5-S₁/R₂₀).

Antibiotic	MIC (µg/ml)	EFSA	EUCAST/ ECOFF	AMR gene
Ampicillin	64	2	4	<i>pbp5-S₁/R₂₀</i>
Vancomycin	1	4	4	-
Gentamycin	32	32	32	<i>aac(6')-li</i>
Kanamycin	>4096	1024	n.a.	<i>aph(3')-III</i>
Streptomycin	>1024	128	128	<i>aad6, aadE</i>
Erythromycin	>512	4	4	<i>ermB, mrsC</i>
Clindamycin	>512	4	n.a.	<i>ermB, lnuB</i>
Tylosine	>512	4	n.a.	<i>ermB</i>
Tetracycline	128	4	4	<i>tetL, tetM</i>
Chloramphenicol	8	16	32	-

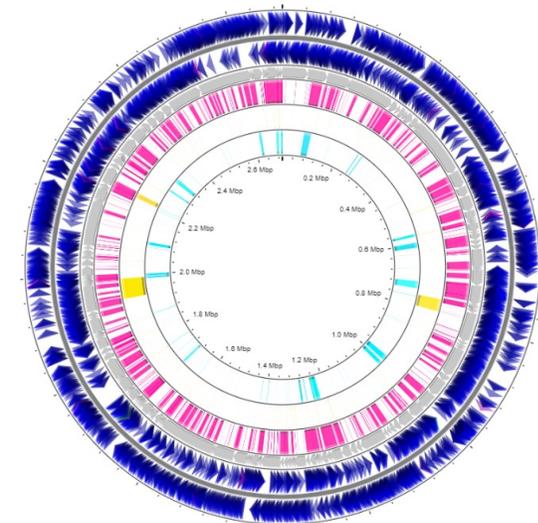


AMR gene exchange in food

- The donor multidrug resistant strain *E. faecium* UC7251 which harbours:
 - *tet(M)* tetracycline resistance gene on the conjugative transposon Tn916.
 - *ermB* erythromycin resistance gene on a conjugative plasmid
- Horizontal gene exchange was assessed in:
 - plate mating experiments (8 recipients species)
 - sausage model (*E. faecalis* as recipient)
- Conjugal transfer of the *ermB* plasmid was observed in *E. faecium*
- Tn 916 interspecific gene exchange occurs in *E. faecalis* and *Lactobacillus rhamnosus*
- Higher transfer frequencies were detected in cheese model (8×10^{-5} transconjugant/donor) than in plate (2×10^{-7} transconjugant/donor)



Accession: 123456
Length: Short



Mobile Genetic Elements



1. The data on AMR in
the food chain

The EU reports

2. Risk assessment of
AMR in food

RTE Foods:
Hazards characterisation
and Exposure assessment

3. The measures for risk
mitigation

The EFSA approach



- reduced use of AMR in animal farming: need for alternatives
- bacterial strains and products intentionally introduced in the food chain should not spread AMR
- Food improvement agents, Feed Additives and Plant Protection Products should not add AMR genes



SCIENTIFIC OPINION

ADOPTED: 1 December 2016 (EFSA BIOHAZ Panel), 8 December 2016 (EMA CVMP)

doi: 10.2903/j.efsa.2017.4666

EMA and EFSA Joint Scientific Opinion on measures to reduce the need to use antimicrobial agents in animal husbandry in the European Union, and the resulting impacts on food safety (RONAFA)

SCIENTIFIC OPINION

ADOPTED: 12 December 2019

doi: 10.2903/j.efsa.2020.5966

Scientific Opinion on the update of the list of QPS-recommended biological agents intentionally added to food or feed as notified to EFSA (2017–2019)

STATEMENT

APPROVED: 2 March 2021

PUBLISHED: 10 March 2021

EFSA statement on the requirements for whole genome sequence analysis of microorganisms intentionally used in the food chain



4.4.	Alternative measures	127
4.4.1.	Organic acids	128
4.4.2.	Probiotics and live microorganisms	129
4.4.2.1.	Probiotics	129
4.4.2.2.	Predatory bacteria	130
4.4.2.3.	Competitive exclusion	130
4.4.3.	Bacteriophages	131
4.4.4.	Prebiotics	132
4.4.5.	Synbiotics	133
4.4.6.	Antibodies	134
4.4.7.	Immunomodulators	135
4.4.8.	Antimicrobial peptides (AMPs)	136
4.4.8.1.	Bacteriocins	136
4.4.8.2.	Host defence peptides	136
4.4.9.	Interferon	137
4.4.10.	Teat sealants	137
4.4.11.	Botanicals	137
4.4.11.1.	Plant feed supplementation	138
4.4.11.2.	Essential oils	138
4.4.12.	Biocides	139
4.4.13.	Clay	139
4.4.14.	Minerals	140
4.4.15.	Other alternatives	141
4.4.16.	Summary on the alternative measures	141



BACTERIAL STRAINS AND PRODUCTS INTENTIONALLY INTRODUCED IN THE FOOD CHAIN SHOULD NOT CONTRIBUTE TO AMR SPREAD

- QPS (Qualified Presumption of Safety) approach – BIOHAZ
- FEED additives guidance – FEEDAP
- FOOD enzymes statement - CEP

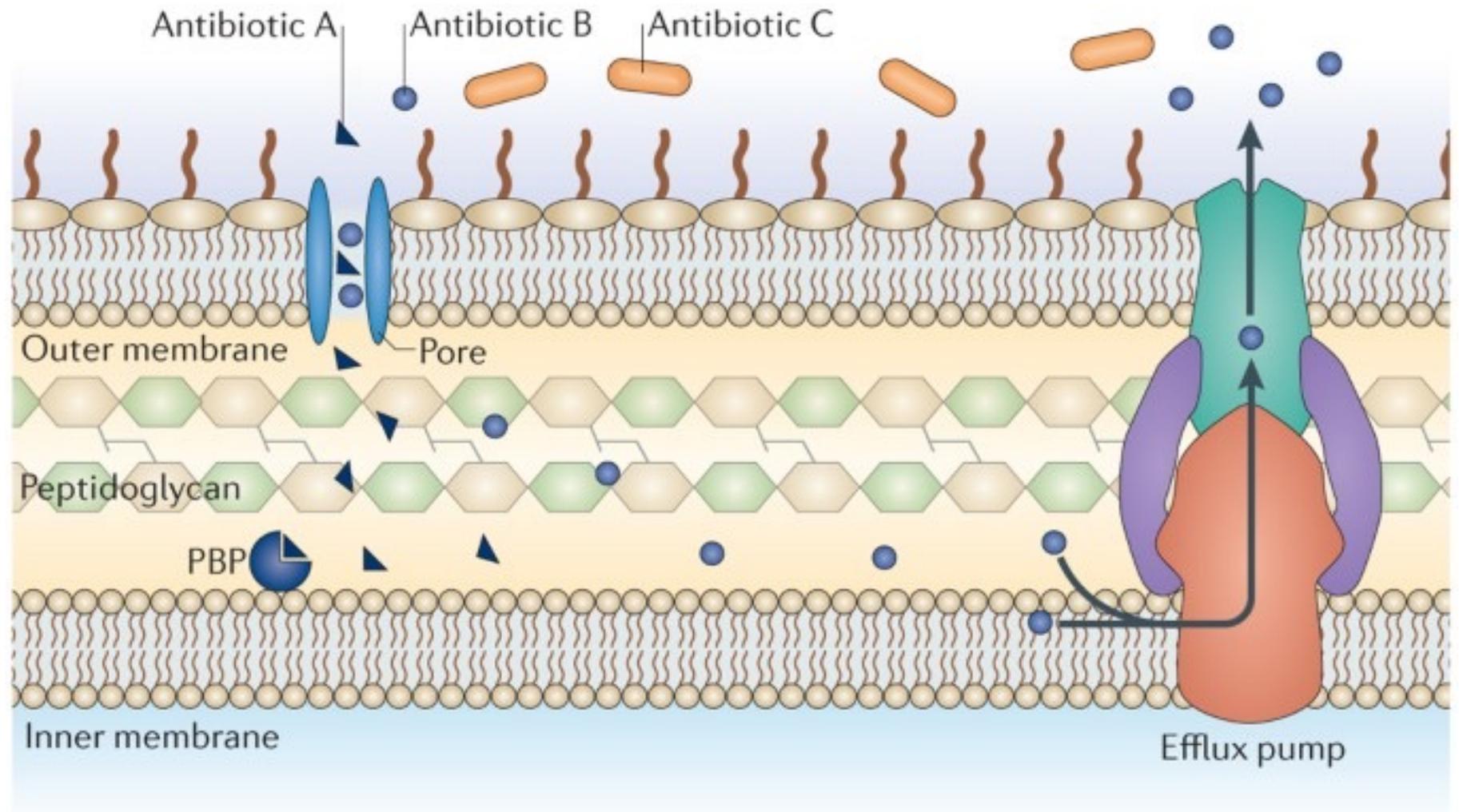
EFSA AMR-susceptibility assessment:

a combined use of **genomic** and **phenotypic** data

Whole Genome Sequence

- presence of genes coding for resistance to antimicrobials relevant to their use in humans and animals (CIAs or HIAs).
- focusing on complete genes coding for resistance to antimicrobials.

Intrinsic Resistance

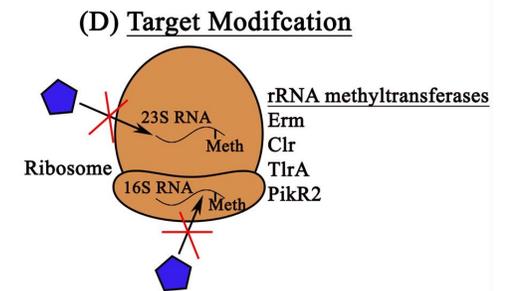
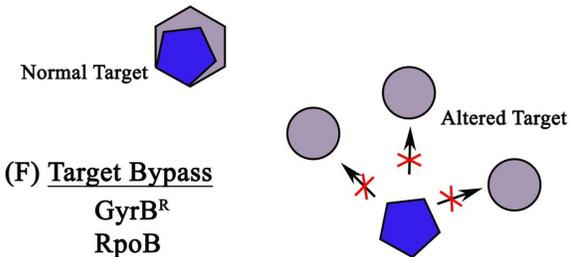
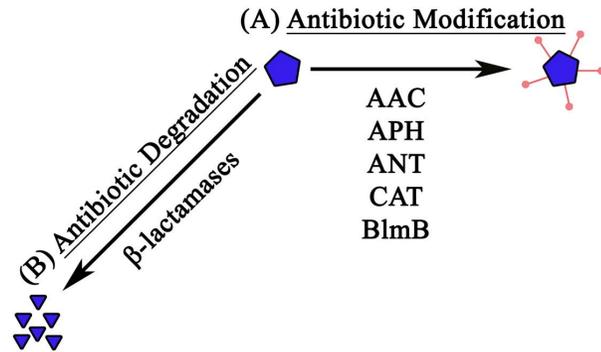
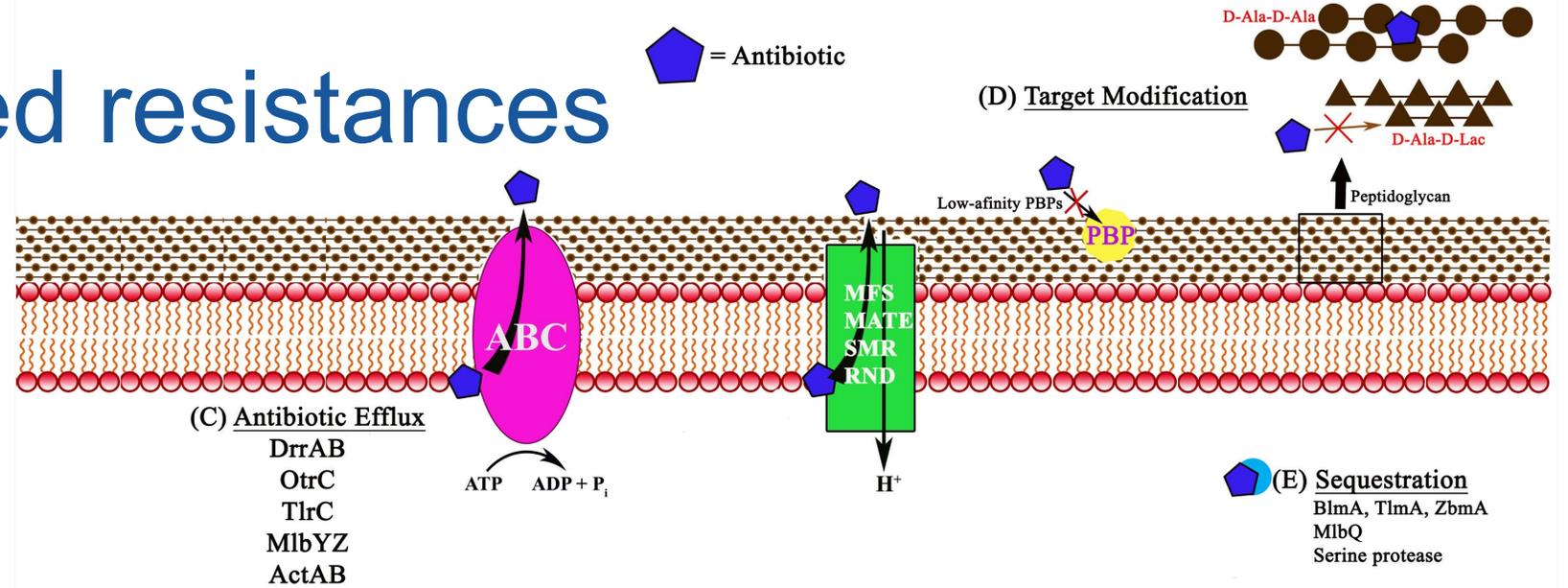


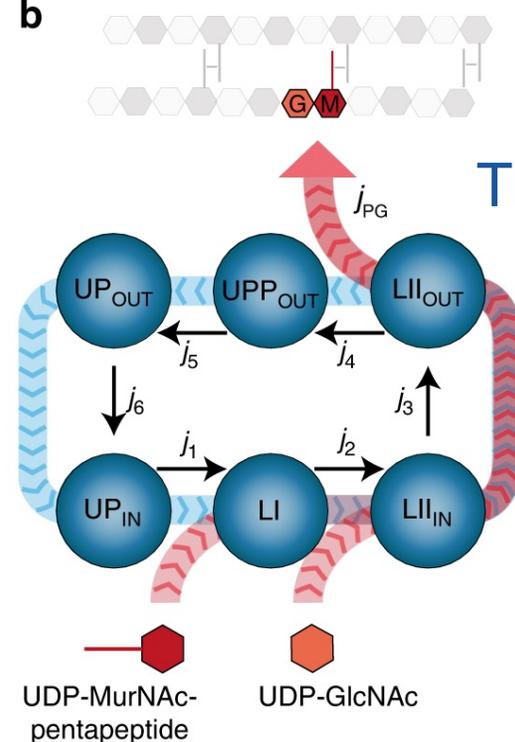
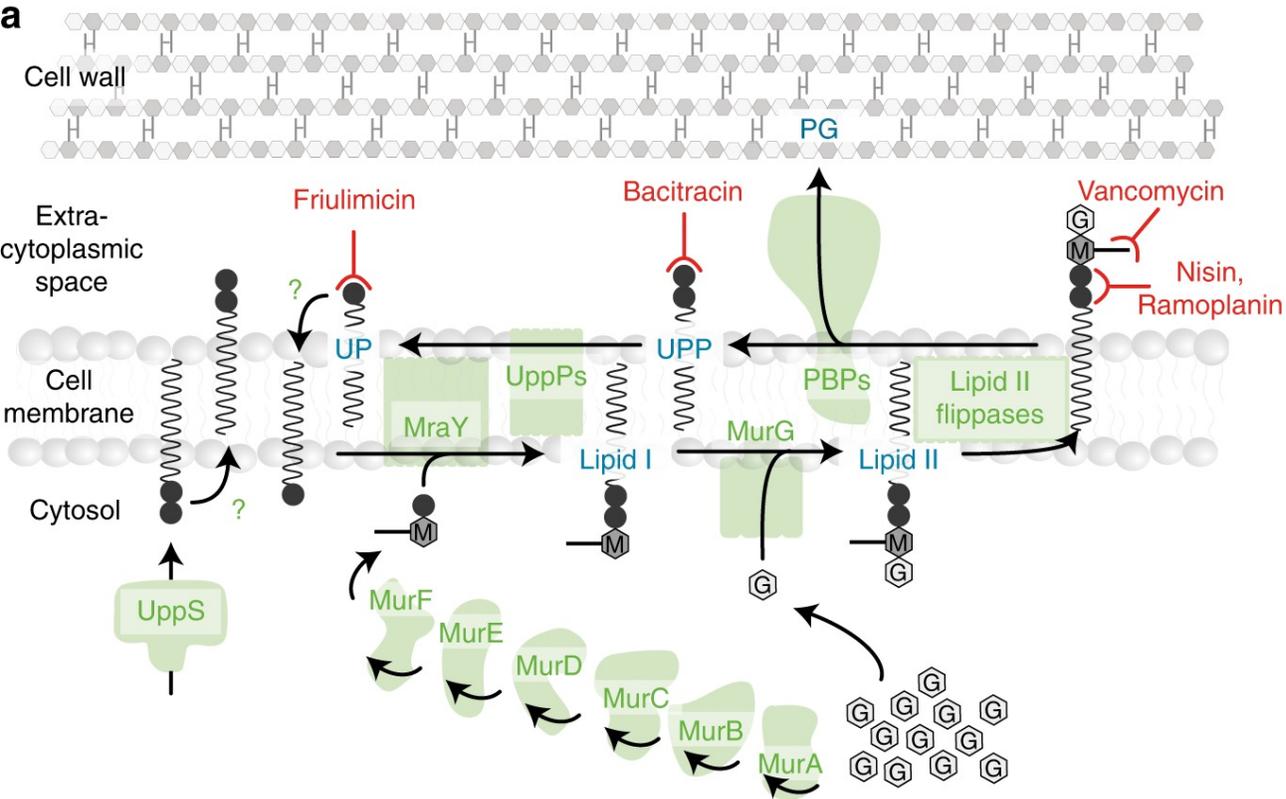
Nature Reviews | **Microbiology**

Blair, J., Webber, M., Baylay, A. *et al.* Molecular mechanisms of antibiotic resistance. *Nat Rev Microbiol* **13**, 42–51 (2015).

<https://doi.org/10.1038/nrmicro3380>

Acquired resistances





Intrinsic vs Acquired The vancomycin resistance

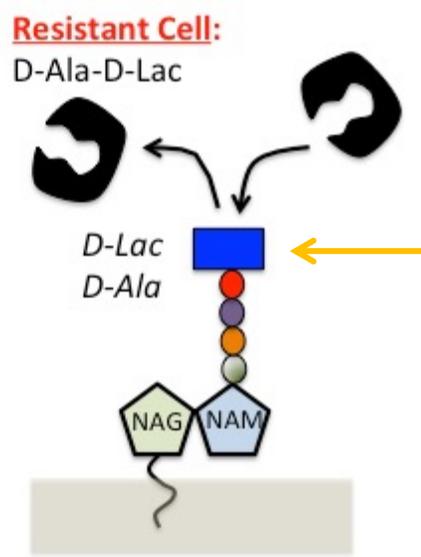
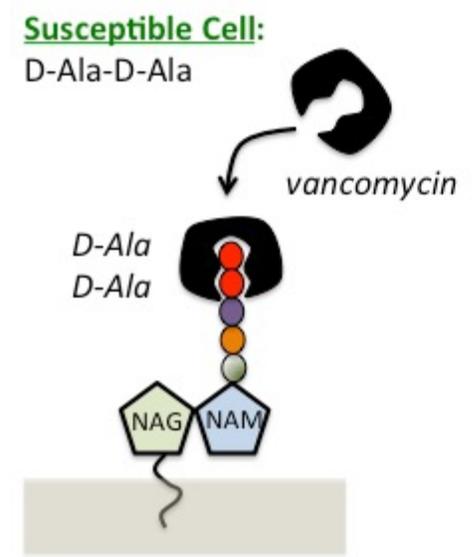
heterofermentative
Lactobacillaceae

Constitutive

D-ala D-lac Ligase

Acquired by HGT

Enterococcus





The data on AMR in the food chain

Still incomplete to achieve a quantitative figure of the AMR flow in the food chain

Risk assessment of AMR in food

Need for more information on the consumer exposure

The measures for risk mitigation

To be developed and applied for RTE foods