



Antibiotico Resistenza nelle filiere alimentari

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AMR in the food chain

1. The data on AMR in the food chain

The EU reports

2. Risk assessment of AMR in food

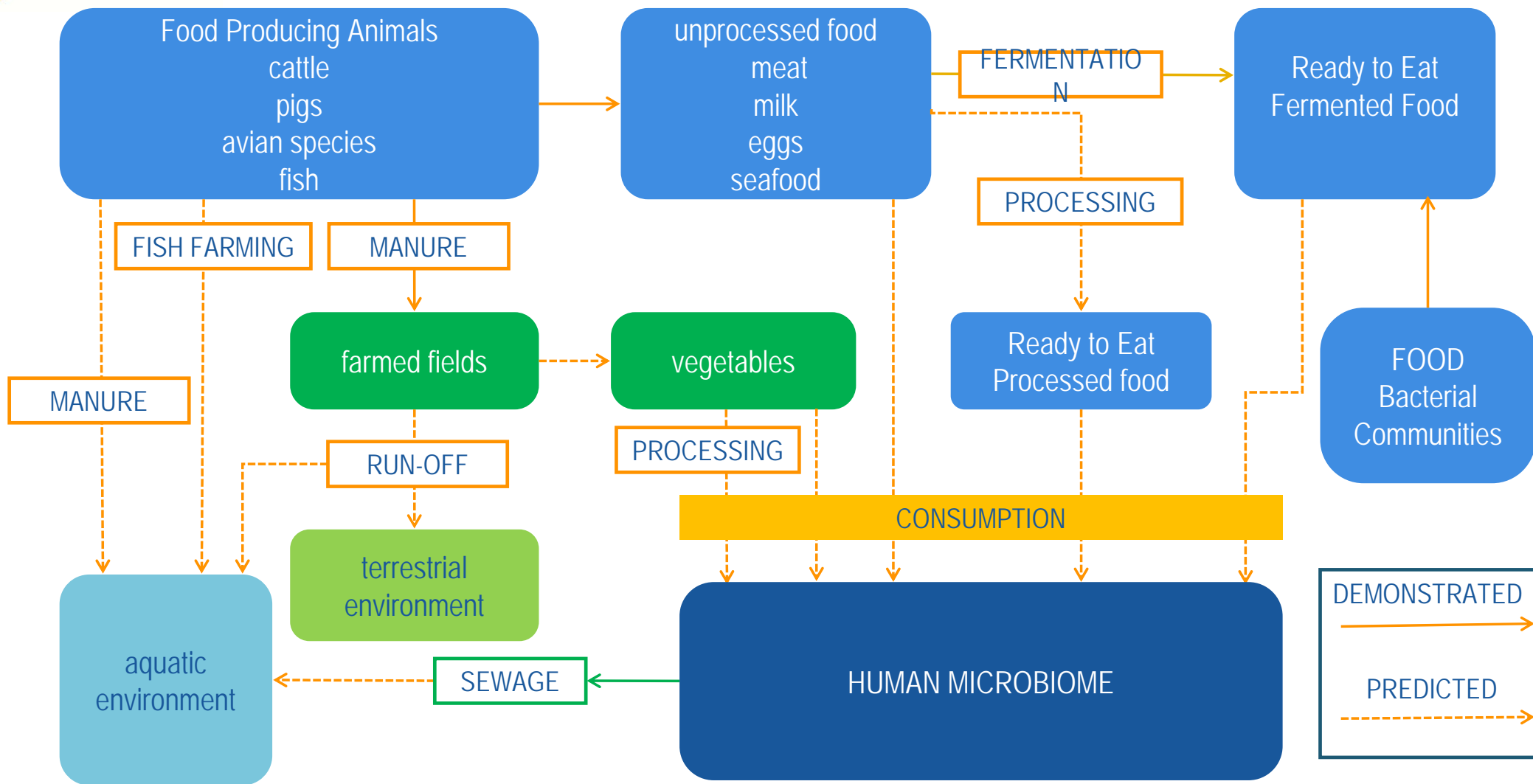
RTE Foods:
Hazards characterisation
and Exposure assessment

3. The measures for risk mitigation

The EFSA approach



FLOW OF AMR BACTERIA/GENES IN THE FOOD CHAIN





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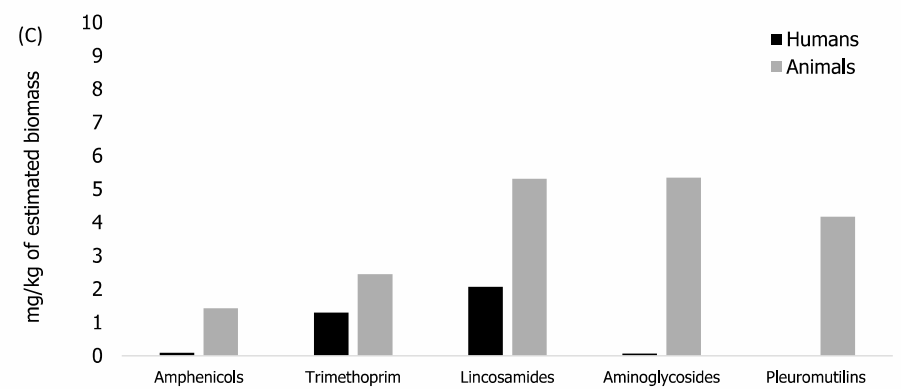
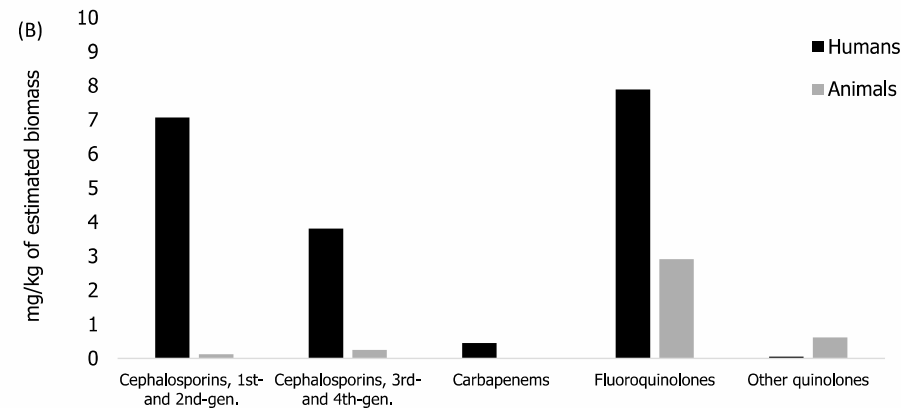
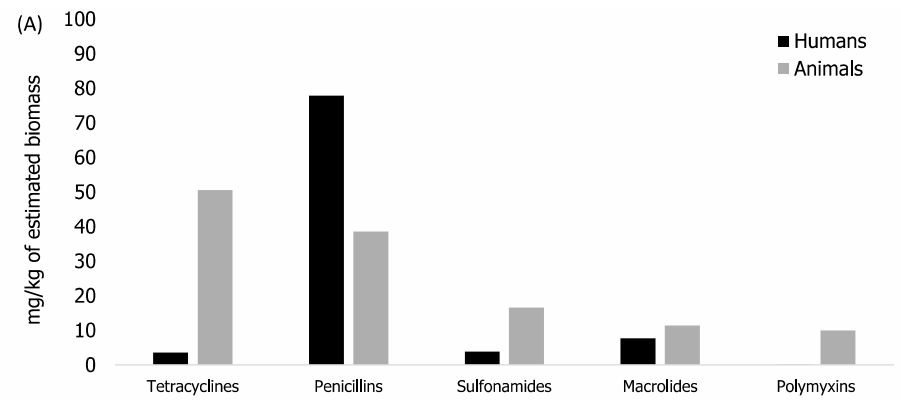
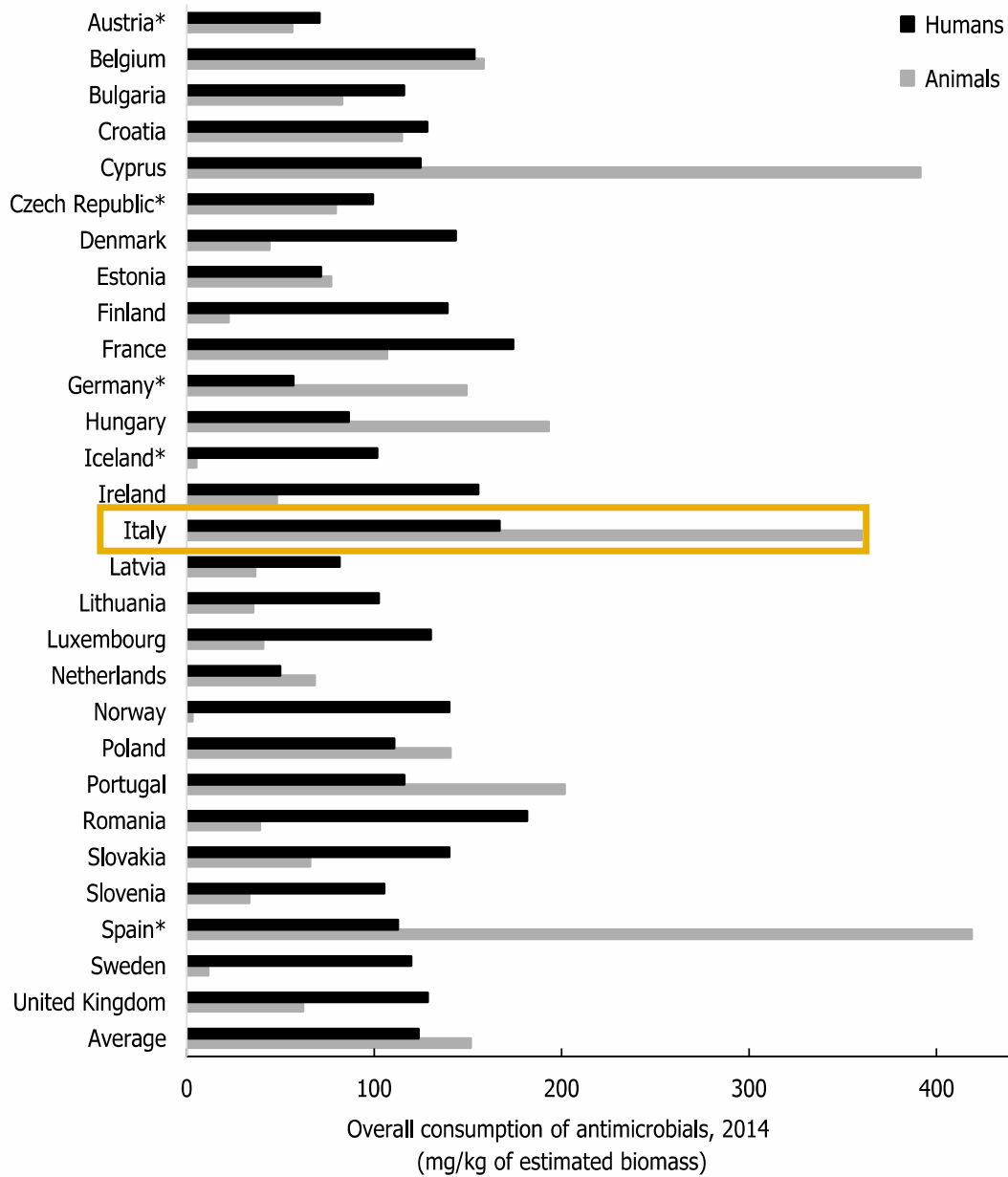
APPROVED: 28 June 2017

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

Joint Interagency Antimicrobial Consumption and Resistance Analysis (JIACRA) Report

European Centre for Disease Prevention and Control (ECDC),
European Food Safety Authority (EFSA) and
European Medicines Agency (EMA)





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SCIENTIFIC REPORT

ADOPTED: 26 January 2017

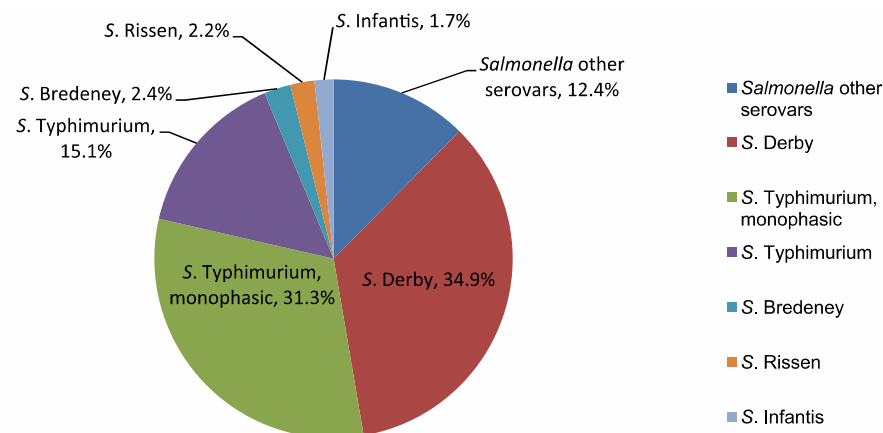
doi: 10.2903/j.efsa.2017.4694

The European Union summary report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2015

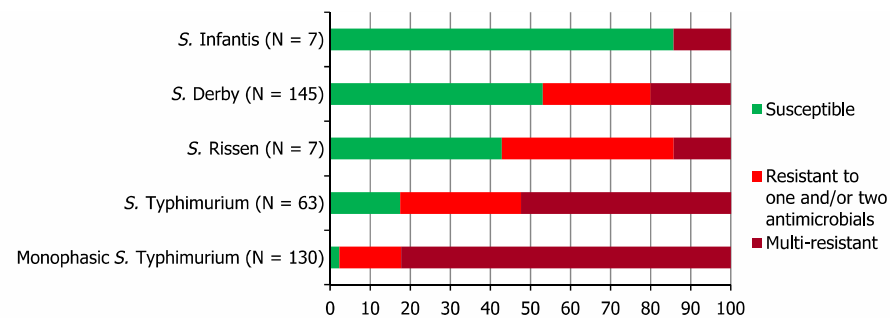
European Food Safety Authority
European Centre for Disease Prevention and Control

Salmonella
Campylobacter
Escherichia coli
MR-*Staphylococcus aureus*

ONLY RAW MEAT DATA



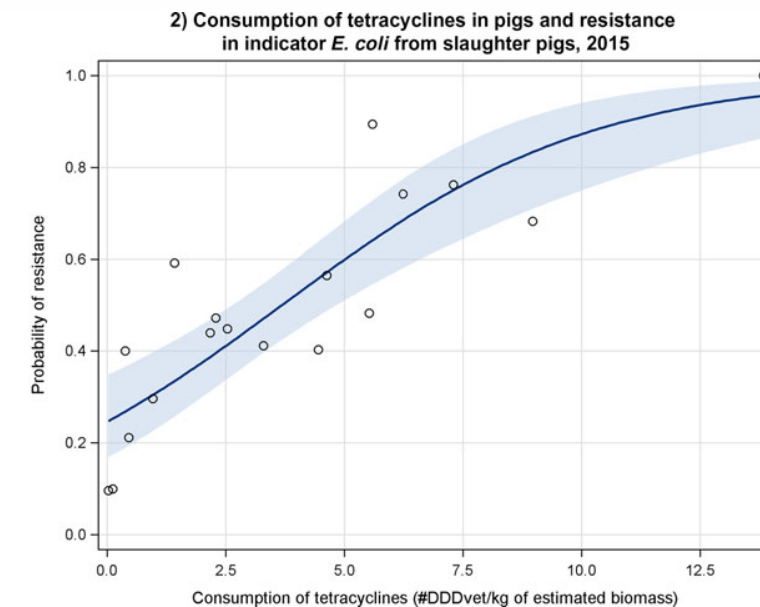
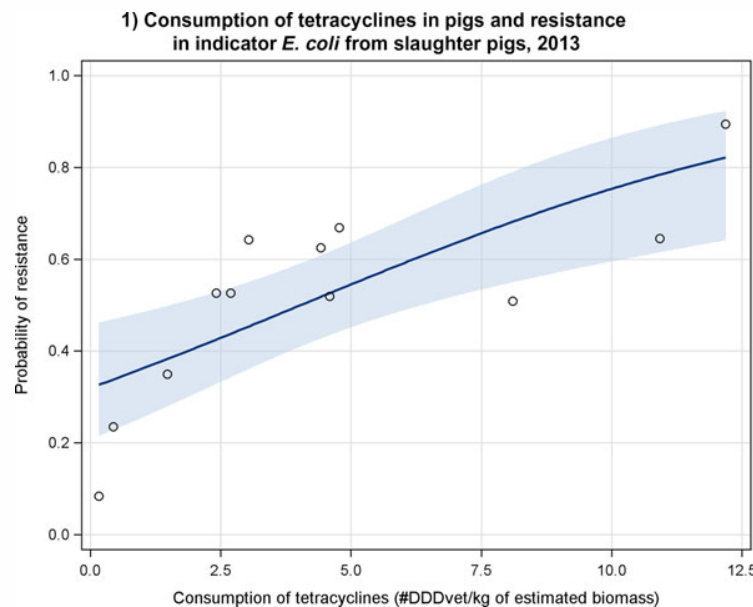
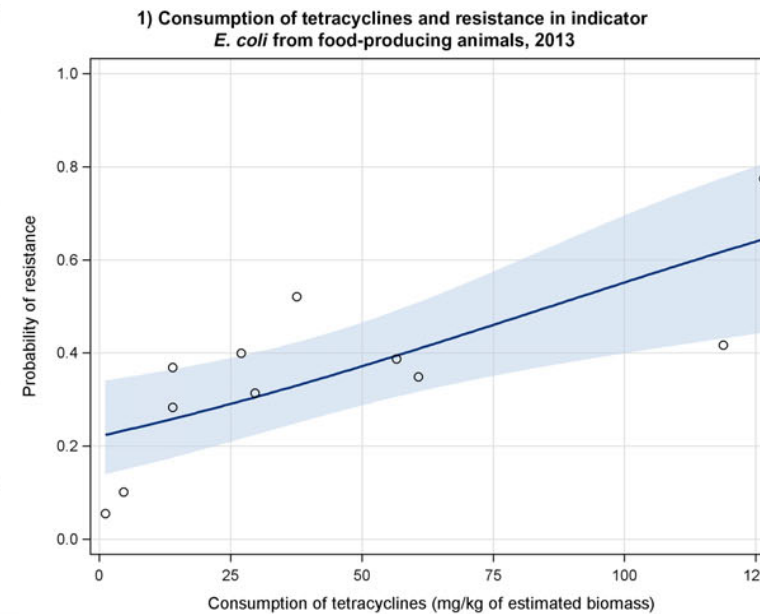
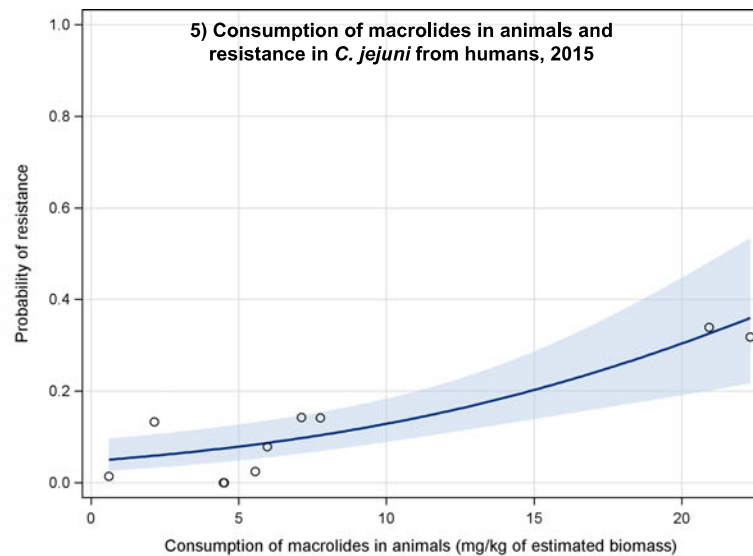
Breakdown of serovars in *Salmonella* isolates from fattening pigs tested for antimicrobial susceptibility in the EU, 2015





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





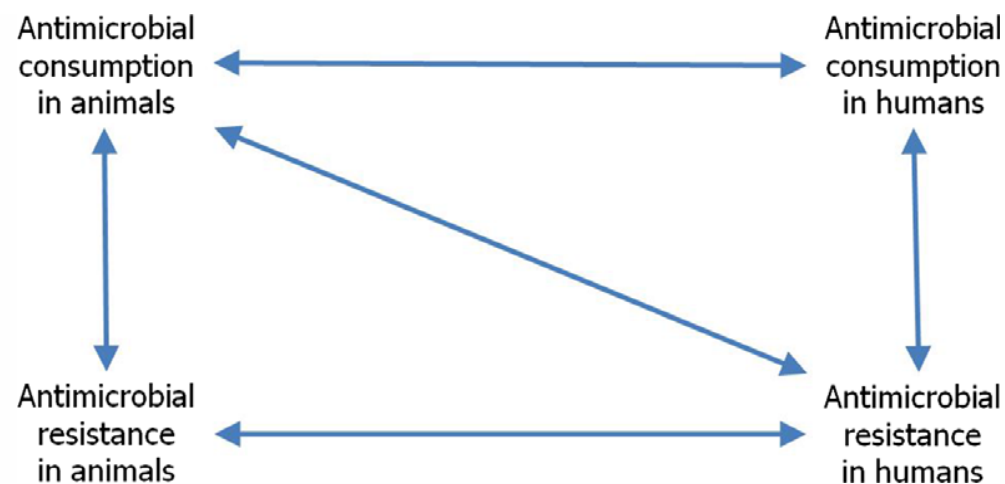
Dots represent countries included in the analysis.

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)



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})

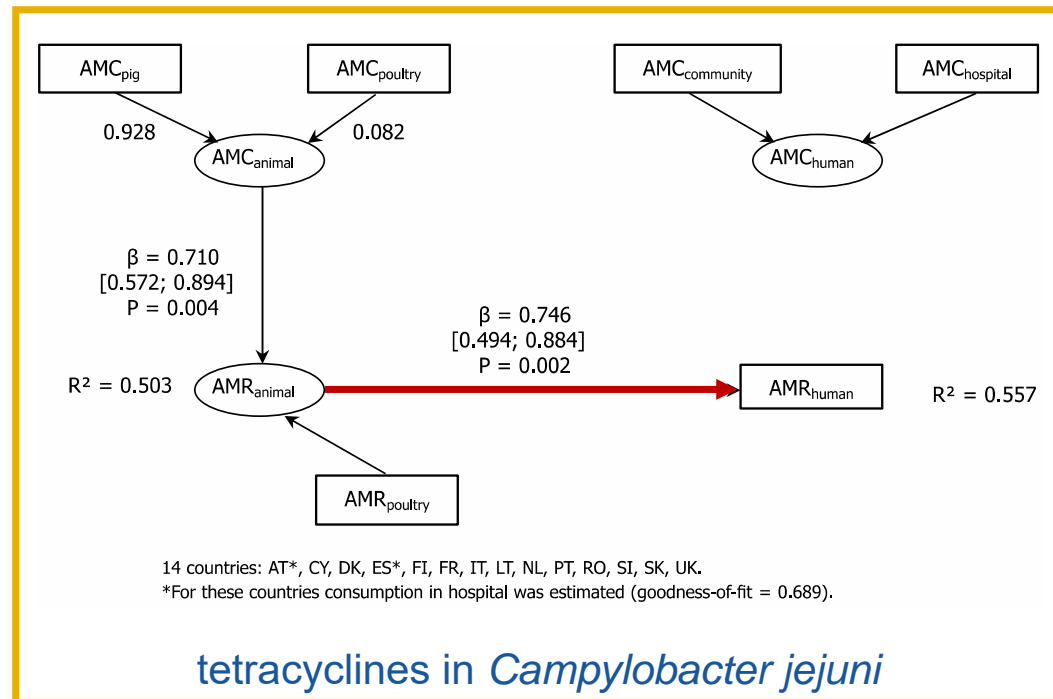
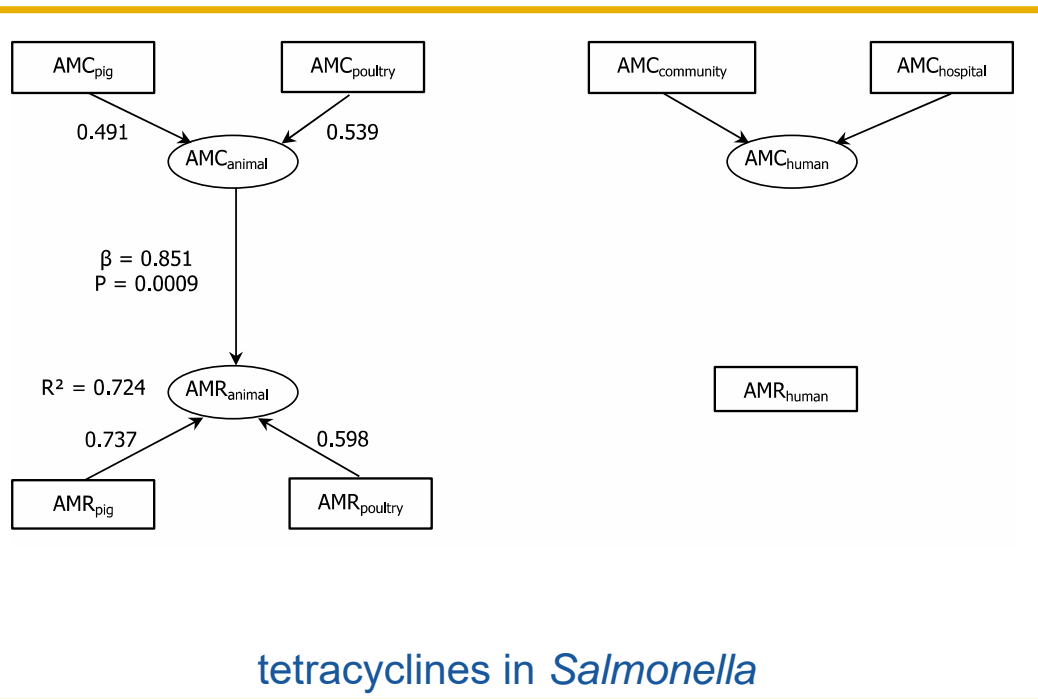


Note: The relationship between AMC in humans and AMR in food-producing animals was not addressed in this report.

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



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})





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



Meticillin-resistant *Staphylococcus aureus* – possible food-borne transmission

Larsen et al., 2016 describe cases of sporadic colonisation or illness in people living in an urban environment in Denmark with a particular type of LA-MRSA, CC9/CC398, *spa*-type t899. The isolates all harboured the Φ Sa3 phage which carried the immune evasion cluster genes *scn* (encoding the staphylococcal complement protein inhibitor), *chp* (chemotaxis inhibitor protein) and *sak* (staphylokinase). Carriage of these genes is considered an adaptation to enable *S. aureus* colonisation and infection of man and is not usually a feature of animal *S. aureus* strains, including LA-MRSA (Cuny et al., 2015). Similar isolates to those detected in humans were detected in poultry and poultry meat and some of the isolates from humans and turkey meat also contained DNA sequences which have been suggested to indicate poultry adaptation. One of the human cases had occupational exposure to meat and another had a brother who was a poultry farmer. These urban LA-MRSA isolates were highly related genetically when epidemiological links between human cases were apparent, suggesting transmission between persons or exposure to a common source. The authors suggest that food-borne transmission was the most probable explanation, at least in some of their reported LA-MRSA t899 cases.

The authors conclude that their findings do not change the generally accepted tenet that food-borne transmission plays a minor role in the epidemiology of LA-MRSA; nevertheless, the study indicates the value of ongoing surveillance of LA-MRSA in animals and food and the benefit of detailed genetic characterisation.



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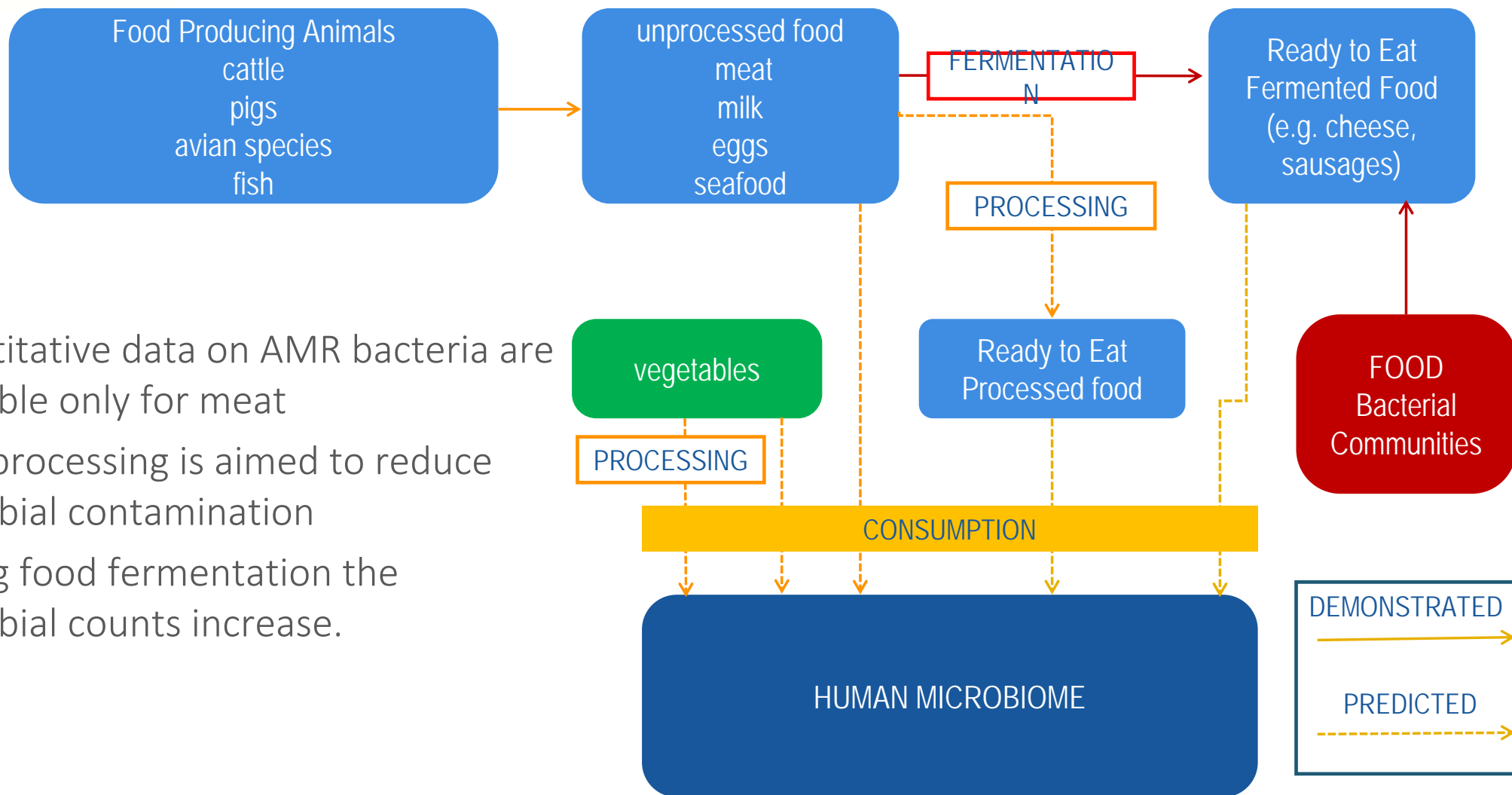
RTE Foods:
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FLOW OF AMR BACTERIA/GENES IN THE FOOD CHAIN



- Quantitative data on AMR bacteria are available only for meat
- food processing is aimed to reduce microbial contamination
- during food fermentation the microbial counts increase.



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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

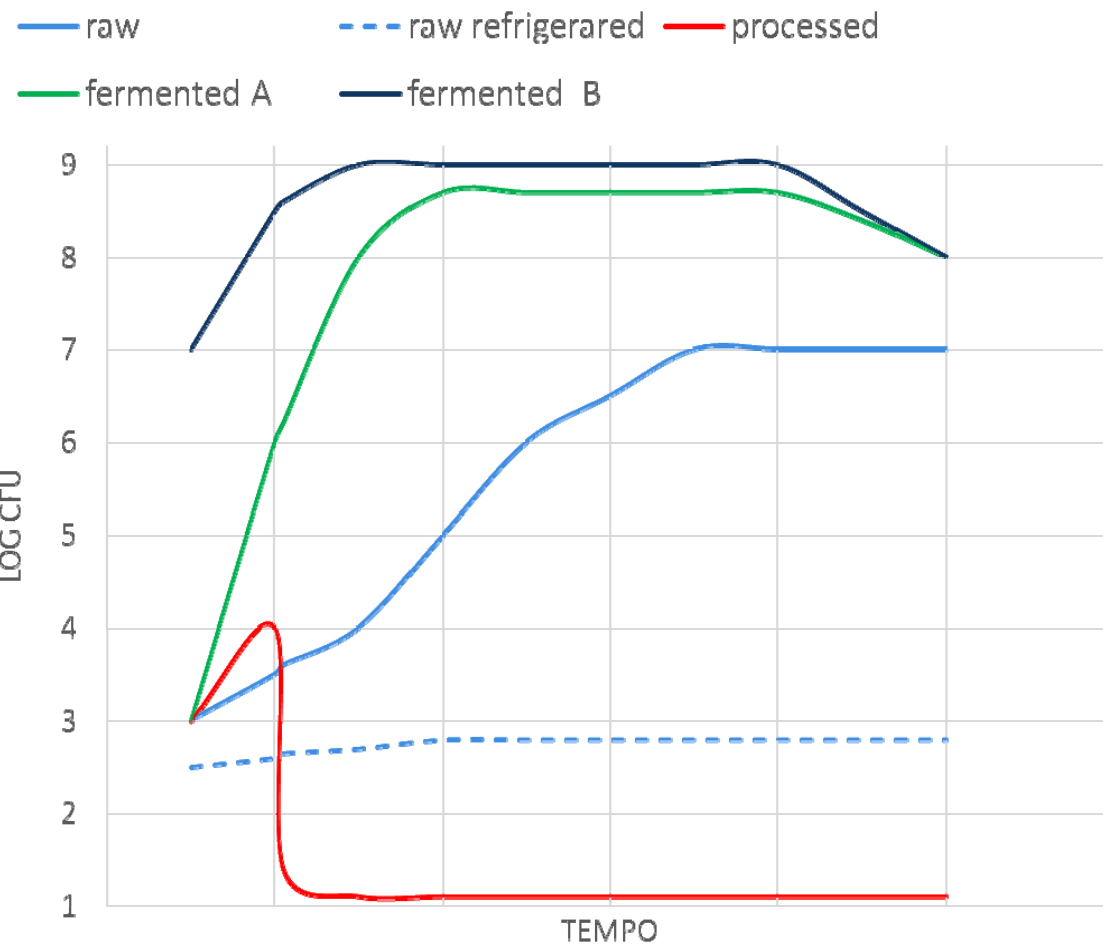
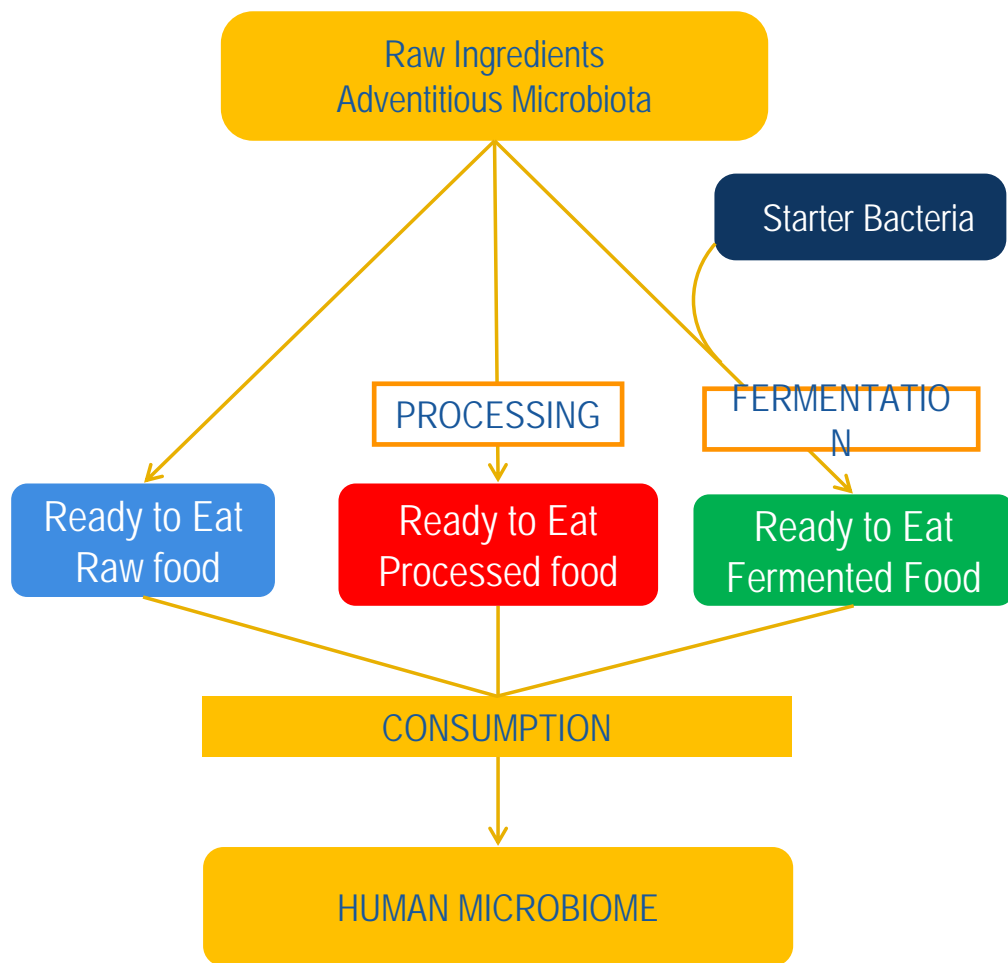
- How many AMR bacteria from RTE foods are ingested?
- What the rate of horizontal gene in food?

Risk Characterization

- What's the risk dimension?



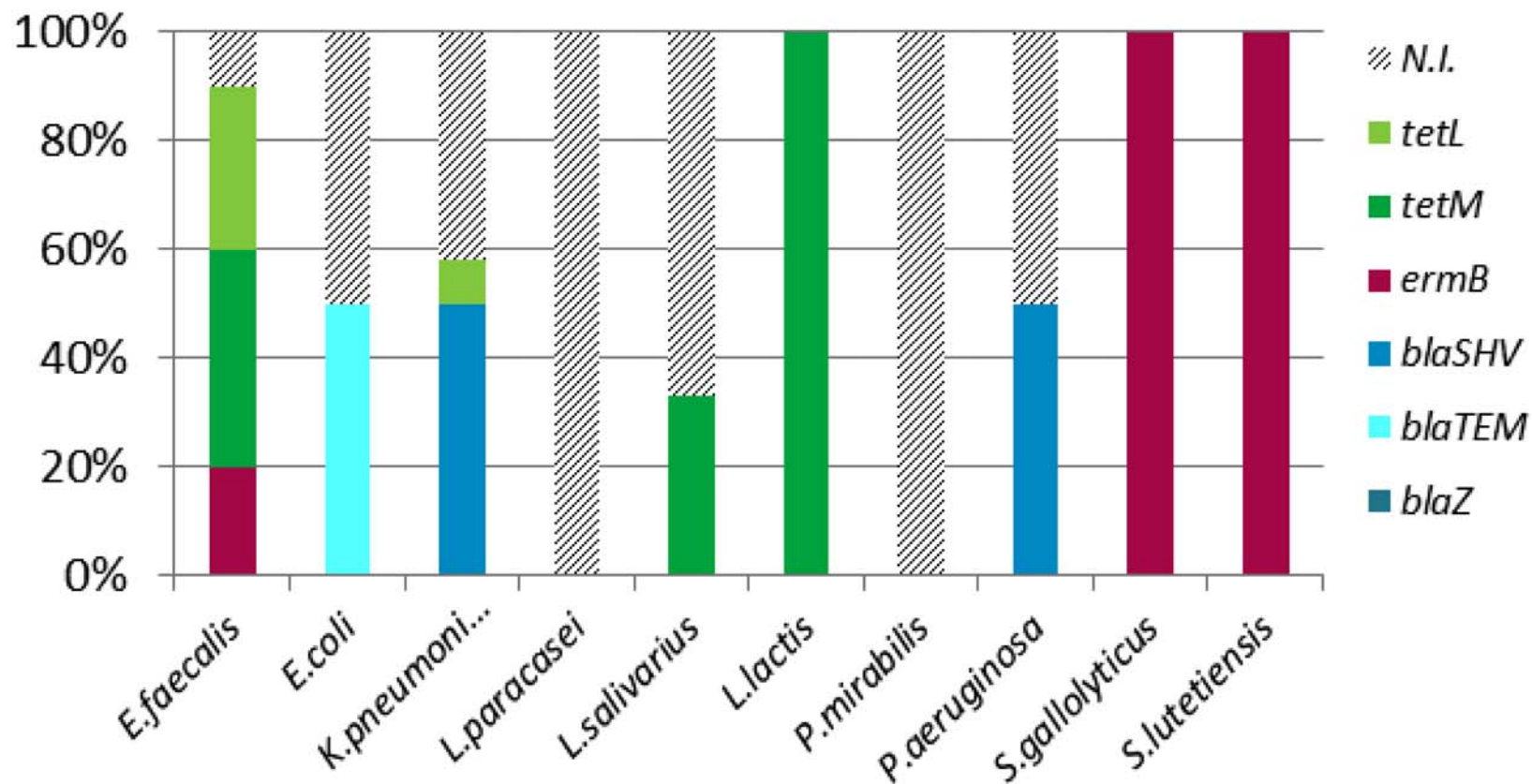
Bacterial Food Community - Consumer Exposure





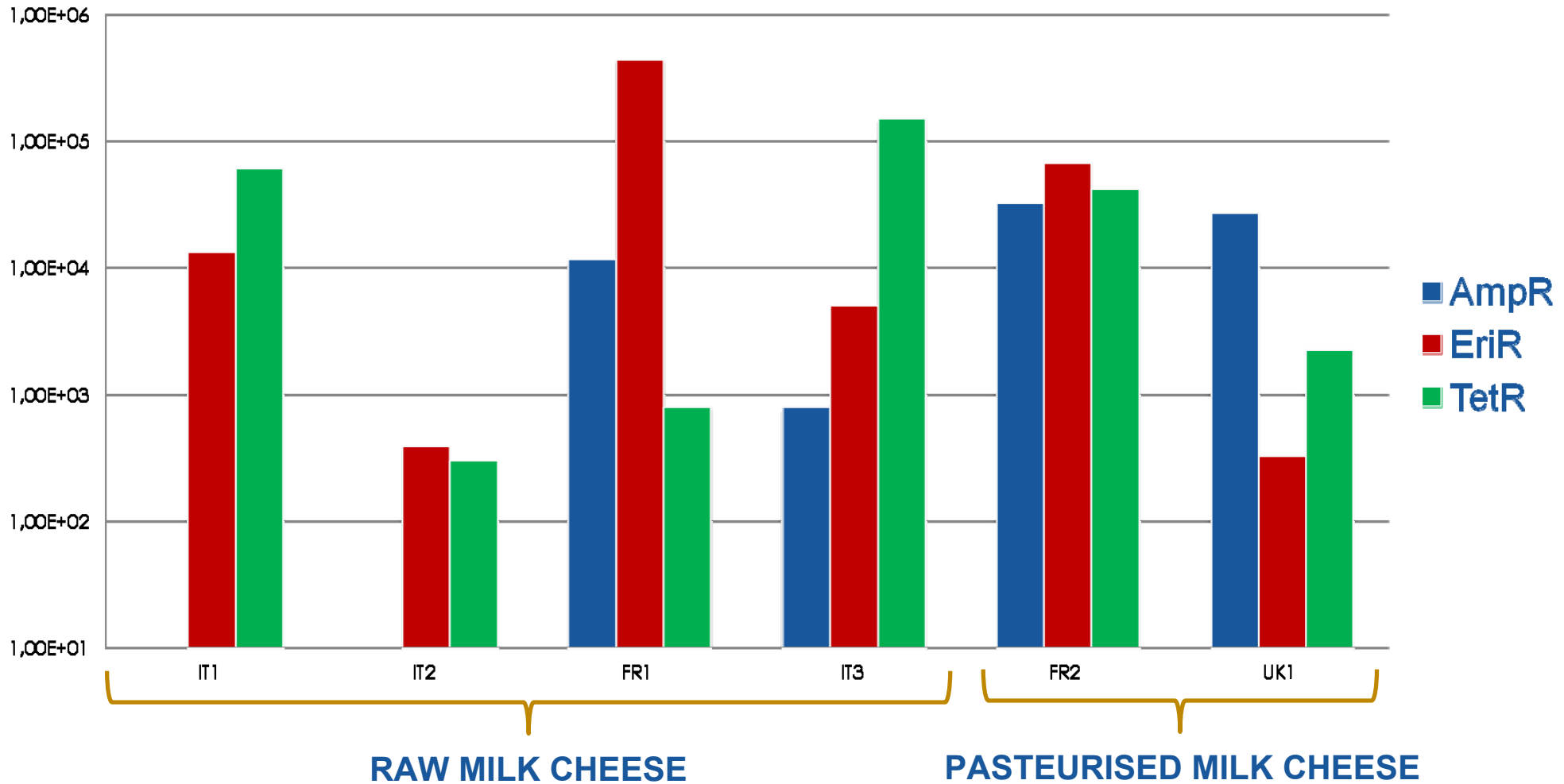
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AMR microbiota in Raw Milk





Prevalence of AMR bacteria in cheese at the consumption stage





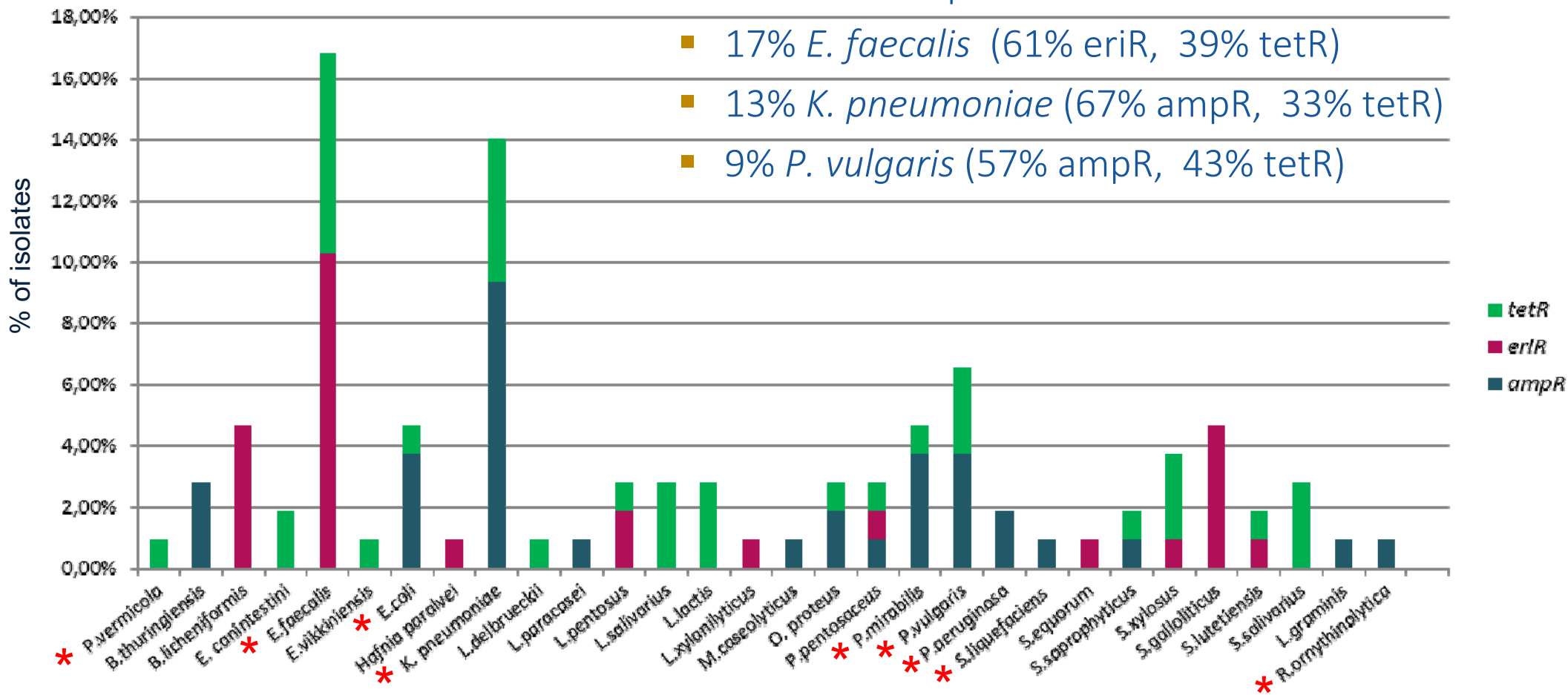
Prevalence of AMR species in dairy samples

213 ISOLATES ■ 30 bacterial species

■ 17% *E. faecalis* (61% *eriR*, 39% *tetR*)

■ 13% *K. pneumoniae* (67% *ampR*, 33% *tetR*)

■ 9% *P. vulgaris* (57% *ampR*, 43% *tetR*)

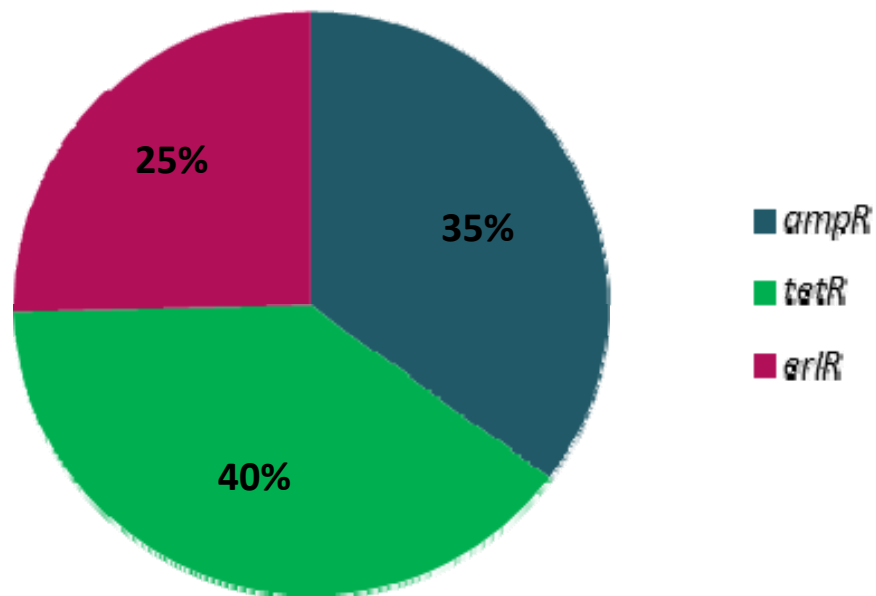


potential human or animal pathogens (*)

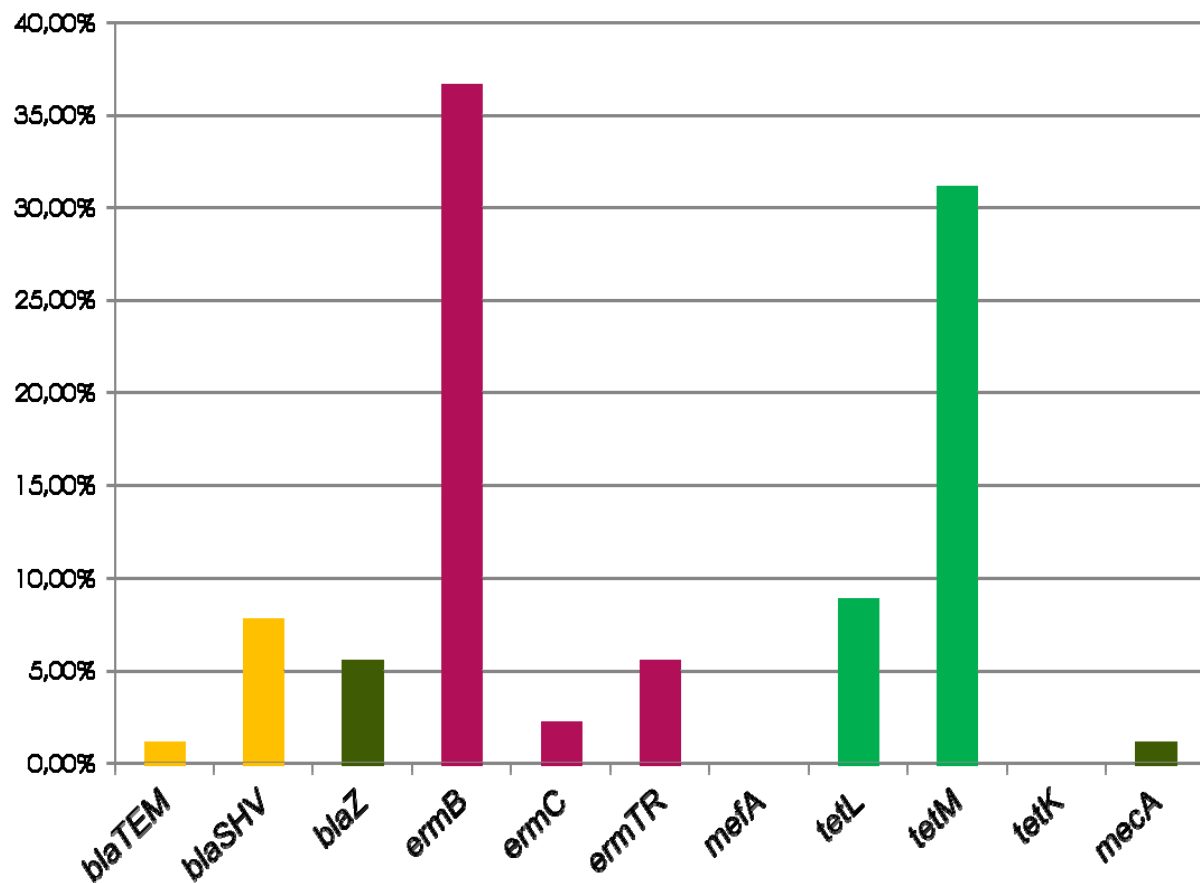


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Prevalence of AMR genotypes in dairy samples



AMR frequency in the total numbers of isolates



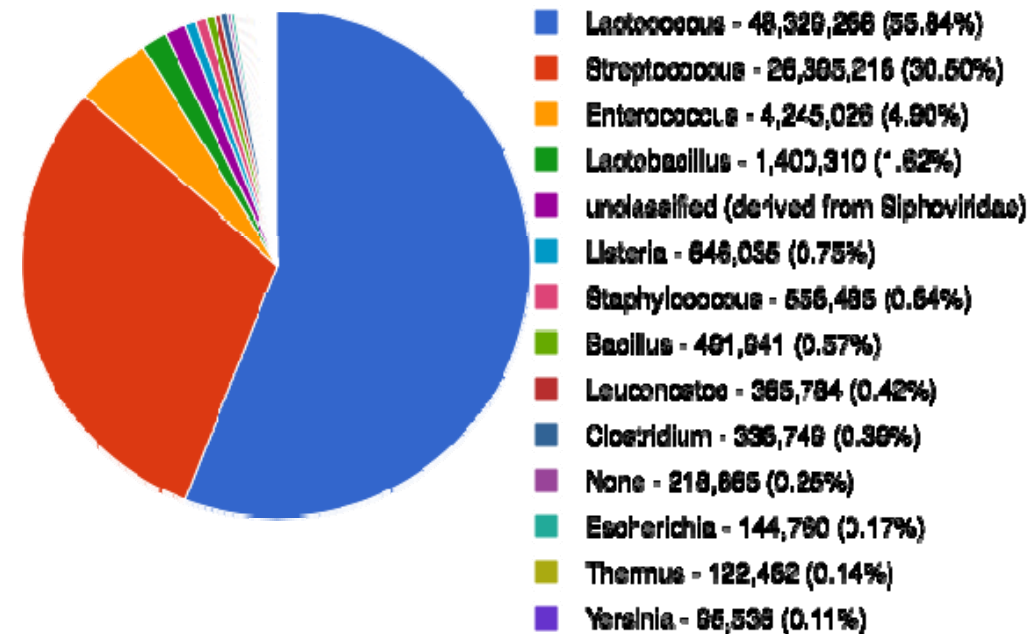
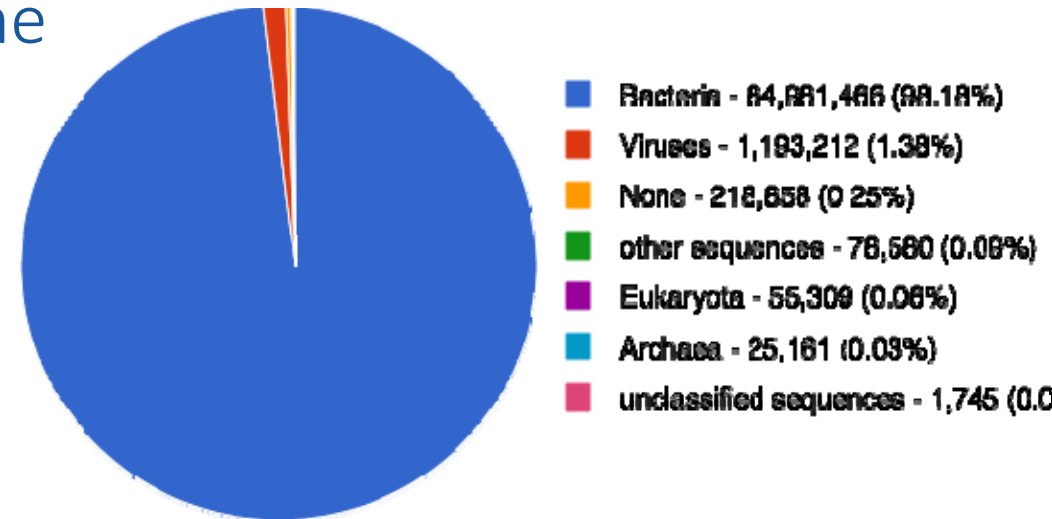
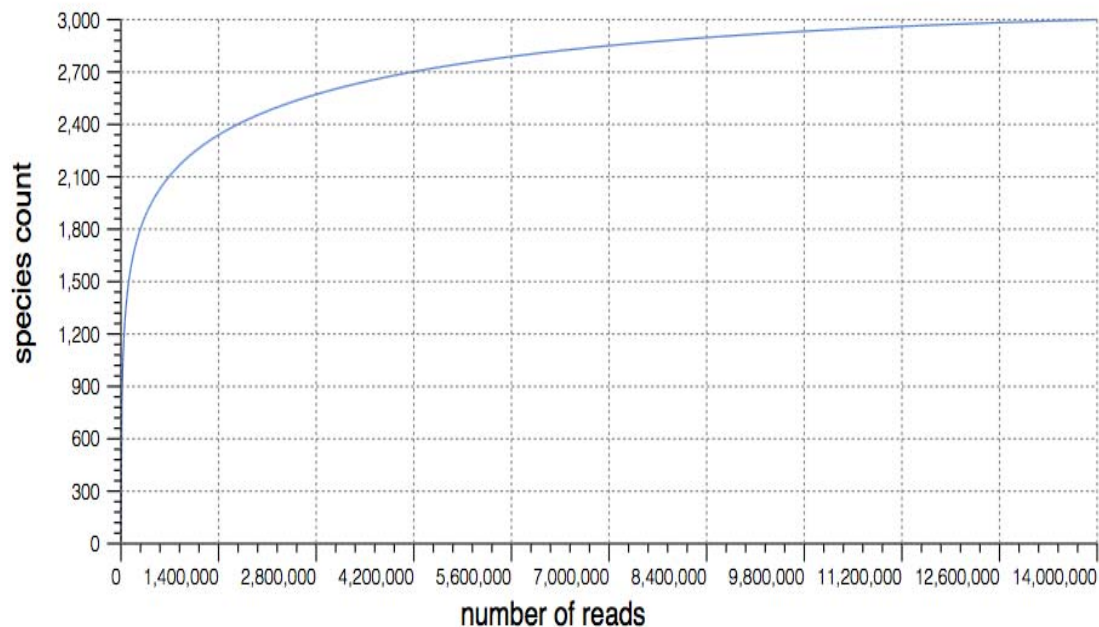
AMR genes distribution in dairy samples



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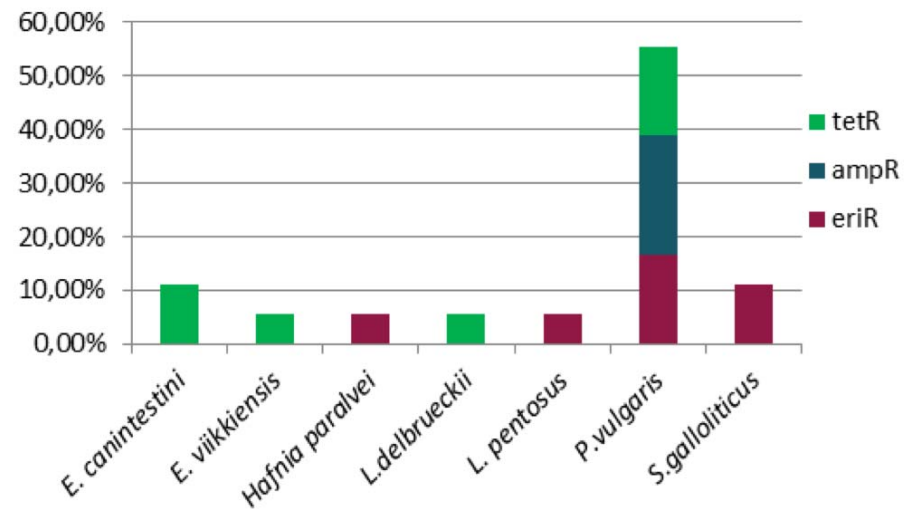
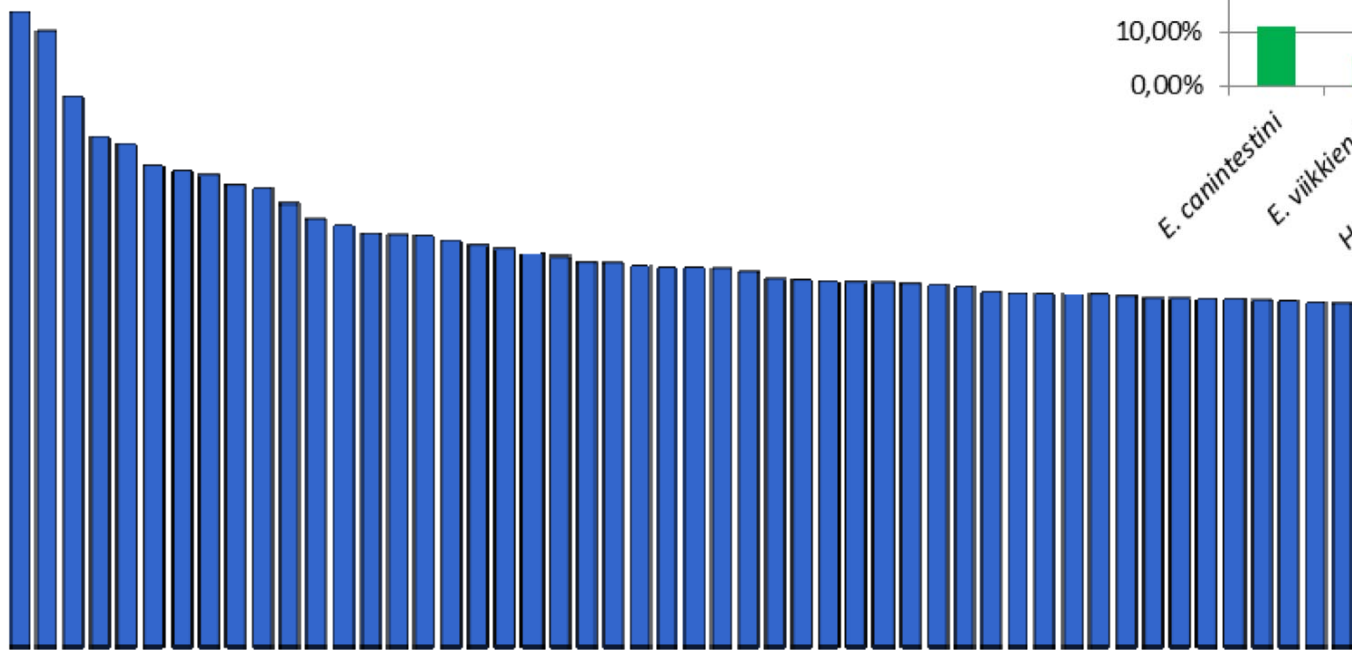
Cheese Metagenome Metaresistome

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





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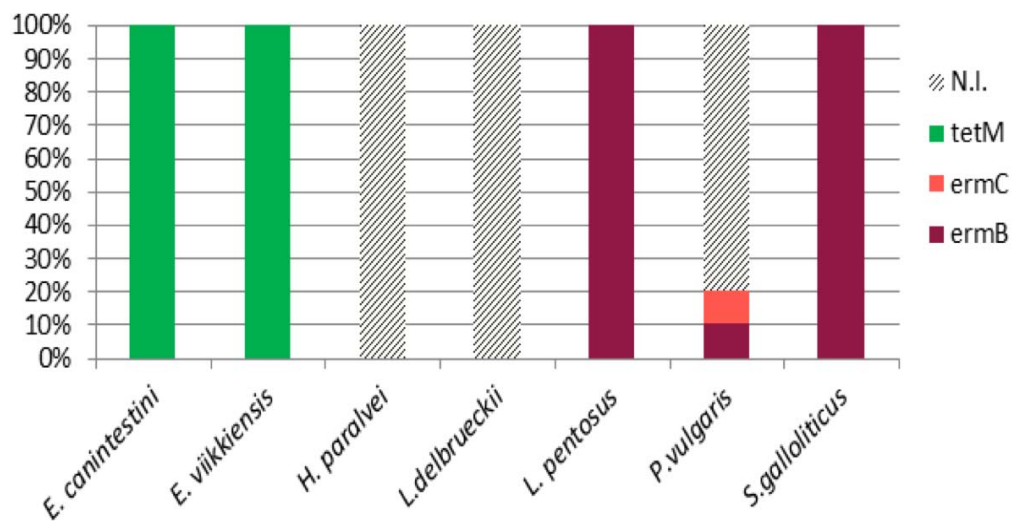


AMR bacterial species identified by cultivation were detected by also by metagenomics



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Cultivation based approach



Meta-resistome Analysis

Center for Genomic Epidemiology

ARO:3000186	tetM	"Enterococcus faecium"
ARO:3000250	ErmC	"Staphylococcus aureus"
ARO:3000375	ErmB	"Streptococcus pneumoniae"
ARO:3000894	TEM-24	"Enterobacter aerogenes"
ARO:3003373	acrR with multidrug antibiotic resistance"	
ARO:3002608	aadA8	"Pseudomonas aeruginosa"
ARO:3002578	AAC(6')-Ib7	"Shigella flexneri"
ARO:3003209	FosA5	"Enterobacter cloacae"
ARO:3002724	QnrB10	"Acinetobacter baumannii"
ARO:3003109	msrE	"Enterobacter cloacae"



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	gen	kan	str	ery	tet	genotype
cut-off	16	64	64	1	8	
IB211		128	128			
IB212	32	256				
IB213		128	128			
IB220					32	tetM
IB231				512		ermB
IB233		128			64	tetK
IB245	32	128				
IB465		512	256			
IB482				512		ermB
IB490					32	tetK
IB491				256		ermC
IB493				512		ermC
IB512		128	128			
IB548		128				
IB612					64	tetM
IB614	32		128			
IB620		128				
IB621				512		ermB
IB622					64	tetM
IB623					32	tetM
IB633		128			64	tetK
IB644		128				
IB652	32	128				
IB654					32	tetK
IB661				512		ermB
IB663				512		ermC
IB665	32	256				
IB666		128				
IB667					64	tetM
IB668					32	tetM
IB678	32			512		ermB
IB702		128				
IB705	32	128				
IB732				512		ermC
IB733			128			
IB742		128				
IB751				512		ermB
IB765				512		ermB
IB782					32	tetK
IB789	32	512				

AMR in *Lactobacillus sakei* strains from fermented sausages

40 strains from fermented meats:

- Tetracycline MIC > cut-off 28%
- Erythromycin MIC > cut-off 28%
- Gentamycin MIC > cut-off 20%
- Kanamycin MIC > cut-off 20%
- Streptomycin MIC > cut-off 48%

None of the strains comply EFSA standard

- Tetracycline resistance
 - *tetM*
 - *tetK*
- Erythromycin resistance
 - *ermB*
 - *ermC*
- High frequency of Aminoglycoside resistance



Resistome prediction in *Staphylococcus. xylosus*

14 genomes – CARD strict algorithm

Strain	Gene	Antimicrobial	WHO category
HKUOPL8	<i>n.d.</i>		
SMQ-121	<i>n.d.</i>		
C2a	<i>n.d.</i>		
S170	<i>fosB3</i>	fosfomycin	CIA
NJ	<i>mphC</i>	macrolide	CIA
LSR_02N	<i>fosB3</i>	fosfomycin	CIA
	<i>mphC</i>	macrolide	CIA
NS341	<i>n.d.</i>		
22B	<i>fosB3</i>	fosfomycin	CIA
CJH_74	<i>nd</i>		
BC10	<i>nd</i>		
733A	<i>tetK</i>	tetracycline	HIA
CHJ_154	<i>fosB3</i>	fosfomycin	CIA
	<i>tetK</i>	tetracycline	CIA
	<i>mphC</i>	macrolide	CIA
DMB3-Bh1	<i>fosB3</i>	fosfomycin	CIA
47-83	<i>mecC</i>	beta-lactam	CIA

mecC

- beta-lactam resistance
- mediates resistance to cefoxitin, oxacillin and methicillin
- low-affinity penicillin-binding protein (PBP) of subclass B1, PBP2c

fosB3

- fosfomycin Resistance
- antibiotic inactivation enzyme
- harboured by mobile elements

mphC

- macrolide resistance
- antibiotic inactivation enzyme

tetK

- tetracycline resistance
- tetracycline efflux protein

Coagulase-negative staphylococci as reservoirs of genes facilitating MRSA infection (Otto, 2014)

BLAST Alignment	<i>mecC</i>
Query	MKKIYISVLVLLIMIIITWLFKDDDIIEKTISSIEKGNVNEVYKNSSEKSKLAYGEEIIV
CARD	MKKIYISVLVLLIMIIITWLFKDDDIIEKTISSIEKGNVNEVYKNSSEKSKLAYGEEIIV
Query	DRNKKIYKDLVNNLKITNHEIKKTKGDKKQVDVKYNIYTKYGTIRRNTQLNFIYEDKHW
CARD	DRNKKIYKDLVNNLKITNHEIKKTKGDKKQVDVKYNIYTKYGTIRRNTQLNFIYEDKHW
Query	KLDWRPDVIVPGLKNGQKINIETLKSERGIKDRNGIELAKTGNTYEIGIVPNKTPKEY
CARD	KLDWRPDVIVPGLKNGQKINIETLKSERGIKDRNGIELAKTGNTYEIGIVPNKTPKEY
Query	DDIARDLQIDTKAITNKVNQKWQVPSFVPIKINKQDEYIDKLIKSYNLQINTIKSRVY
CARD	DDIARDLQIDTKAITNKVNQKWQVPSFVPIKINKQDEYIDKLIKSYNLQINTIKSRVY
Query	PLNEATVHLLGYVGPINSDELKSKQFRNYSKNTVIGKGLERLYDKQLQNTDGFKVSIA
CARD	PLNEATVHLLGYVGPINSDELKSKQFRNYSKNTVIGKGLERLYDKQLQNTDGFKVSIA
Query	TYDNKPLDTLLEKKAENKDLHLTIDARVQESYIKHMKNDGSGTALQPKTGEILALVST
CARD	TYDNKPLDTLLEKKAENKDLHLTIDARVQESYIKHMKNDGSGTALQPKTGEILALVST
Query	PSYDVYPFMNGLSNNDYRKL TNNKKEPLLNFQITTSFGSTQKILTSIALKENKLDKNT
CARD	PSYDVYPFMNGLSNNDYRKL TNNKKEPLLNFQITTSFGSTQKILTSIALKENKLDKNT
Query	NFDIYGKGWQKQDASWGNYNITRFKVVGDGIDLKQAISSDNIFFARIALALGAKKFEQGM
CARD	NFDIYGKGWQKQDASWGNYNITRFKVVGDGIDLKQAISSDNIFFARIALALGAKKFEQGM
Query	QDLGIGENIPSDYPFYKAQISNSLNKNEILLADSGYGQGEILVNP IQILSIYSALENNGN
CARD	QDLGIGENIPSDYPFYKAQISNSLNKNEILLADSGYGQGEILVNP IQILSIYSALENNGN
Query	IQNPHVLRKTKSQIWKDIIIPKDDIDILTNGMERVNVNKRHRDDIYKNYARIIGKSGTAE
CARD	IQNPHVLRKTKSQIWKDIIIPKDDIDILTNGMERVNVNKRHRDDIYKNYARIIGKSGTAE

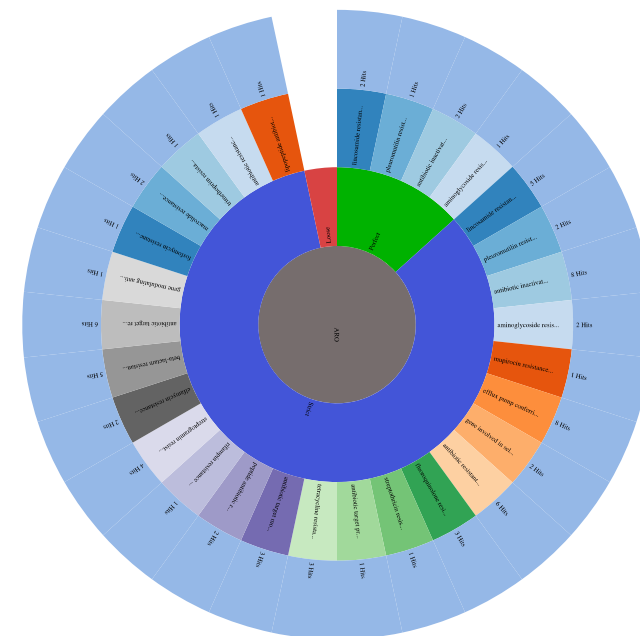


The multidrug resistant *E. faecium* UC7251 isolated from fermented sausage

Resistance	MIC (µg/ml)
Ampicillin	64
Gentamycin	32
Kanamycin	>4096
Streptomycin	>1024
Erythromycin	>512
Clindamycin	>512
Tetracycline	>64

- Multidrug resistant strain
- isolated from fermented sausage
- 12 acquired resistance genes were detected
- *tetM* is harboured by Tn916
- ampicillin resistance: allelic variation in *pbp5* gene
- *ermB* is plasmid coded

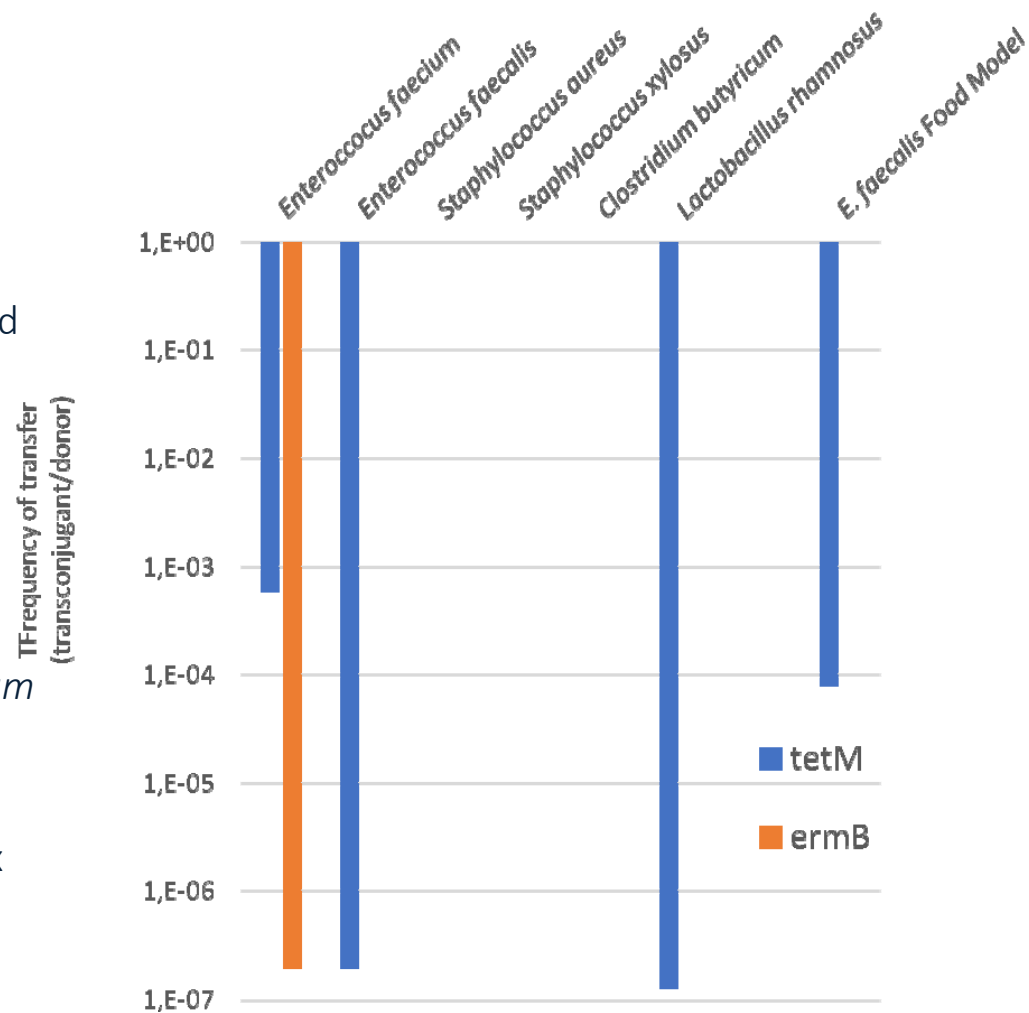
Antibiotic	Acquired Resistance Genotypes			
tetracyclines	<i>tetM</i>	<i>tetK</i>	<i>adeC</i>	
amynoglycosides	<i>APH(3')-IIIa</i>	<i>ANT(9)-Ia</i>	<i>AAC(6')-I</i>	
macrolides	<i>ermB</i>	<i>msrC</i>		
lincosamides	<i>lmrD</i>	<i>lsaA</i>	<i>lmrC</i>	<i>saIA</i>
ampicillin	<i>pbp5-S1/R20</i>			





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)
 - cheese 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)





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MEASURES FOR RISK MITIGATION IN THE FOOD CHAIN

- reduced use of AM in animal farming: need for alternatives
- bacterial strains intentionally introduced in the food chain should not harbour acquired resistance genes



EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH



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: 30 November 2016

doi: 10.2903/j.efsa.2017.4664

Scientific Opinion on the update of the list of QPS-recommended biological agents intentionally added to food or feed as notified to EFSA*



EFSA AMR-susceptibility assessment: a combined use of genomic and phenotypic data

Susceptible: growth is inhibited at a concentration of a specific antimicrobial equal to or lower than the established cut-off value
($S \leq x$ mg/L)

Resistant: growth is not inhibited at a concentration of a specific antimicrobial equal to or lower than the established cut-off value
($R > x$ mg/L)

	ampicillin	vancomycin	gentamicin	kanamycin	streptomycin	erythromycin	clindamycin	tetracycline	chloramphenicol	tylosine	ciprofloxacin	colistine	fosfomicin
<i>Lactobacillus</i> obligate homofermentative ^a	2	2	16	16	16	1	4	4	4	n.r.	n.r.	n.r.	n.r.
<i>Lactobacillus acidophilus</i> group	1	2	16	64	16	1	4	4	4	n.r.	n.r.	n.r.	n.r.
<i>Lactobacillus</i> obligate heterofermentative ^b	2	n.r.	16	64	64	1	4	8 ^c	4	n.r.	n.r.	n.r.	n.r.
<i>Lactobacillus reuteri</i>	2	n.r.	8	64	64	1	4	32	4	n.r.	n.r.	n.r.	n.r.
<i>Lactobacillus</i> facultative heterofermentative ^d	4	n.r.	16	64	64	1	4	8	4	n.r.	n.r.	n.r.	n.r.
<i>Lactobacillus plantarum/pentosus</i>	2	n.r.	16	64	n.r.	1	4	32	8	n.r.	n.r.	n.r.	n.r.
<i>Lactobacillus rhamnosus</i>	4	n.r.	16	64	32	1	4	8	4	n.r.	n.r.	n.r.	n.r.
<i>Lactobacillus casei /paracasei</i>	4	n.r.	32	64	64	1	4	4	4	n.r.	n.r.	n.r.	n.r.
<i>Bifidobacterium</i>	2	2	64	n.r.	128	1	1	8	4	n.r.	n.r.	n.r.	n.r.
<i>Pediococcus</i>	4	n.r.	16	64	64	1	1	8	4	n.r.	n.r.	n.r.	n.r.
<i>Leuconostoc</i>	2	n.r.	16	16	64	1	1	8	4	n.r.	n.r.	n.r.	n.r.
<i>Lactococcus lactis</i>	2	4	32	64	32	1	1	4	8	n.r.	n.r.	n.r.	n.r.
<i>Streptococcus thermophilus</i>	2	4	32	n.r.	64	2	2	4	4	n.r.	n.r.	n.r.	n.r.
<i>Bacillus</i>	n.r.	4	4	8	8	4	4	8	8	n.r.	n.r.	n.r.	n.r.
<i>Propionibacterium</i>	2	4	64	64	64	0.5	0.25	2	2	n.r.	n.r.	n.r.	n.r.
<i>Enterococcus faecium</i>	2	4	32	1024	128	4	4	4	16	4	n.r.	n.r.	n.r.
<i>Corynebacterium</i> and Other Gram +	1	4	4	16	8	1	4	2	4	n.r.	n.r.	n.r.	n.r.
Enterobacteriaceae	8	n.r.	2	8	16	n.r.	n.r.	8	n.r.	n.r.	0.06	2	8

n.r. not required.

^a including *L. delbrueckii*, *L. helveticus*

^b including *L. fermentum*

^c for *L. buchneri* the cut-off for tetracycline is 128

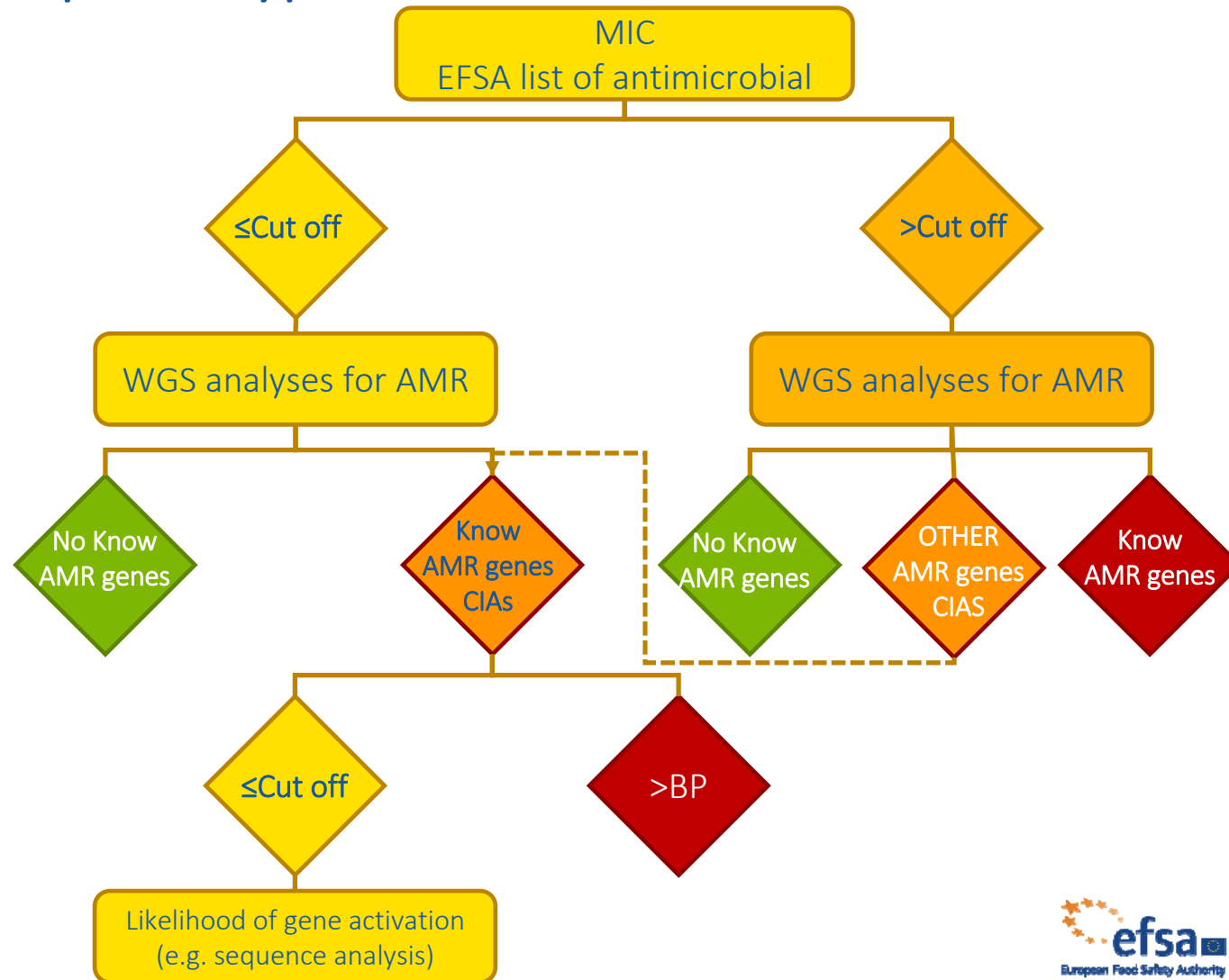
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^d including the homofermentative species *L. salivarius*



EFSA AMR-susceptibility assessment: a combined use of genomic and phenotypic data

- WGS interrogation
- Presence of genes coding for resistance to antimicrobials relevant to their use in humans and animals (CIAs or HIAs).
- Data focusing on complete genes coding for resistance to antimicrobials.
- Data should include at least the gene identification, function of the encoded protein, percentage of identity and e-value.





- The data on AMR in the food chain Still incomplete to achieve a quantitative figure of the AMR flow in the of food chain
- Risk assessment of AMR in food Need for more information on the consumer exposure
- The measures for risk mitigation To be developed for RTE foods