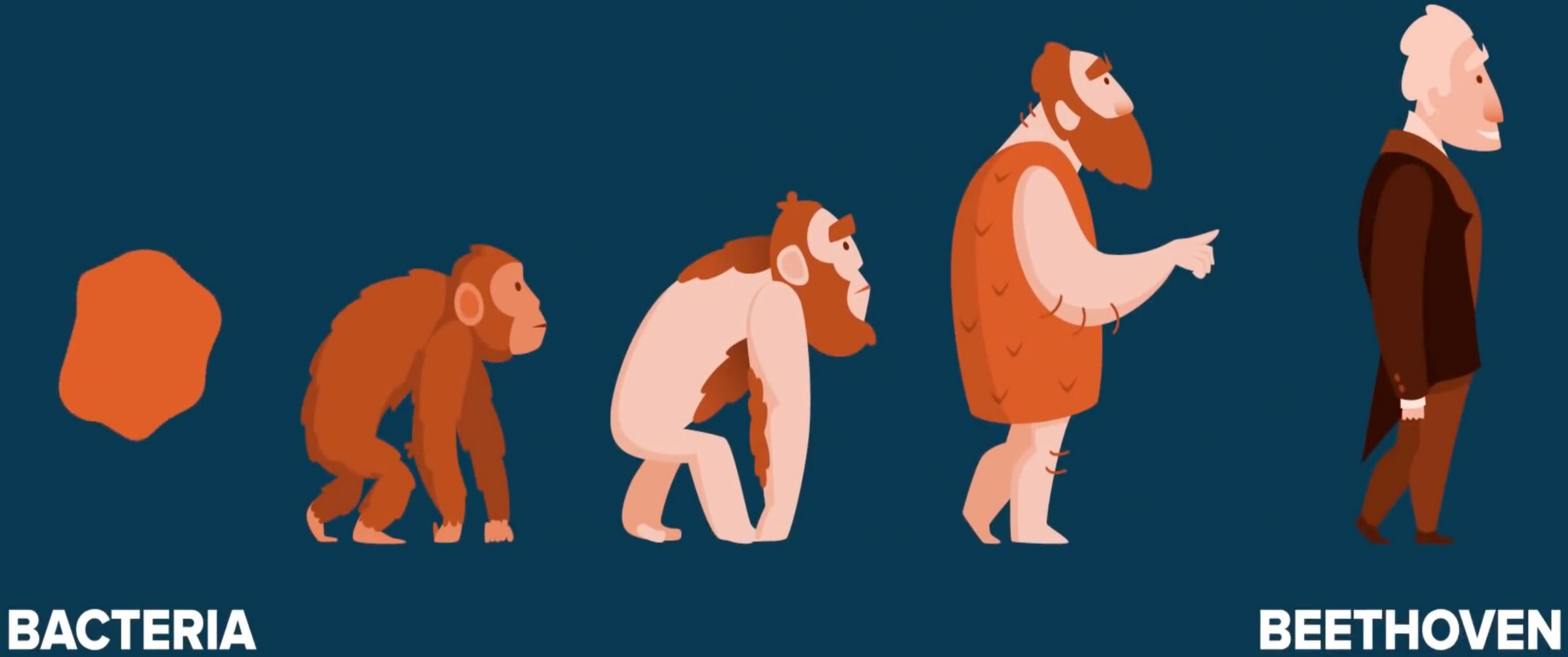
Impact of antimicrobial resistance on poultry gut health and metabolism

Speaker: mzaghari@ut.ac.ir
Available at **www.minatoyoor.com**


BACTERIA
BEETHOVEN

Outline of presentation

- Antibiotics:
  - Mode of action and mechanisms of resistance
  - Genetic basis of antimicrobial resistance
  - Applied approach (disc diffusion, PCR, . . ., metagenomics)
  - How does resistance spread?
- Human:
  - GLASS (global antimicrobial resistance surveillance system)
  - Antibiotic use, resistance and the link to human nutrition
- Poultry:
  - Antibiotic application in poultry production
  - Antibiotic resistance of some selected organisms in poultry
  - Antibiotic use, resistance and the link to poultry nutrition

Paul Ehrlich

Appearance of antimicrobial resistance

Antibiotic resistance plasmids

VRE

ESBL

MRSA

Penicillinase

Natural dyes
Heavy metal

1900 1910 1920 1930 1940 1950 1960 1970 1980 1990 2000 2010 2020

Pre-antibiotic era

Penicillin was discovered in 1928

Sulfonamides
Penicillin

Aminoglycosides,
Chloramphenicol,
Tetracycline,
Macrolide

Cephalosporins

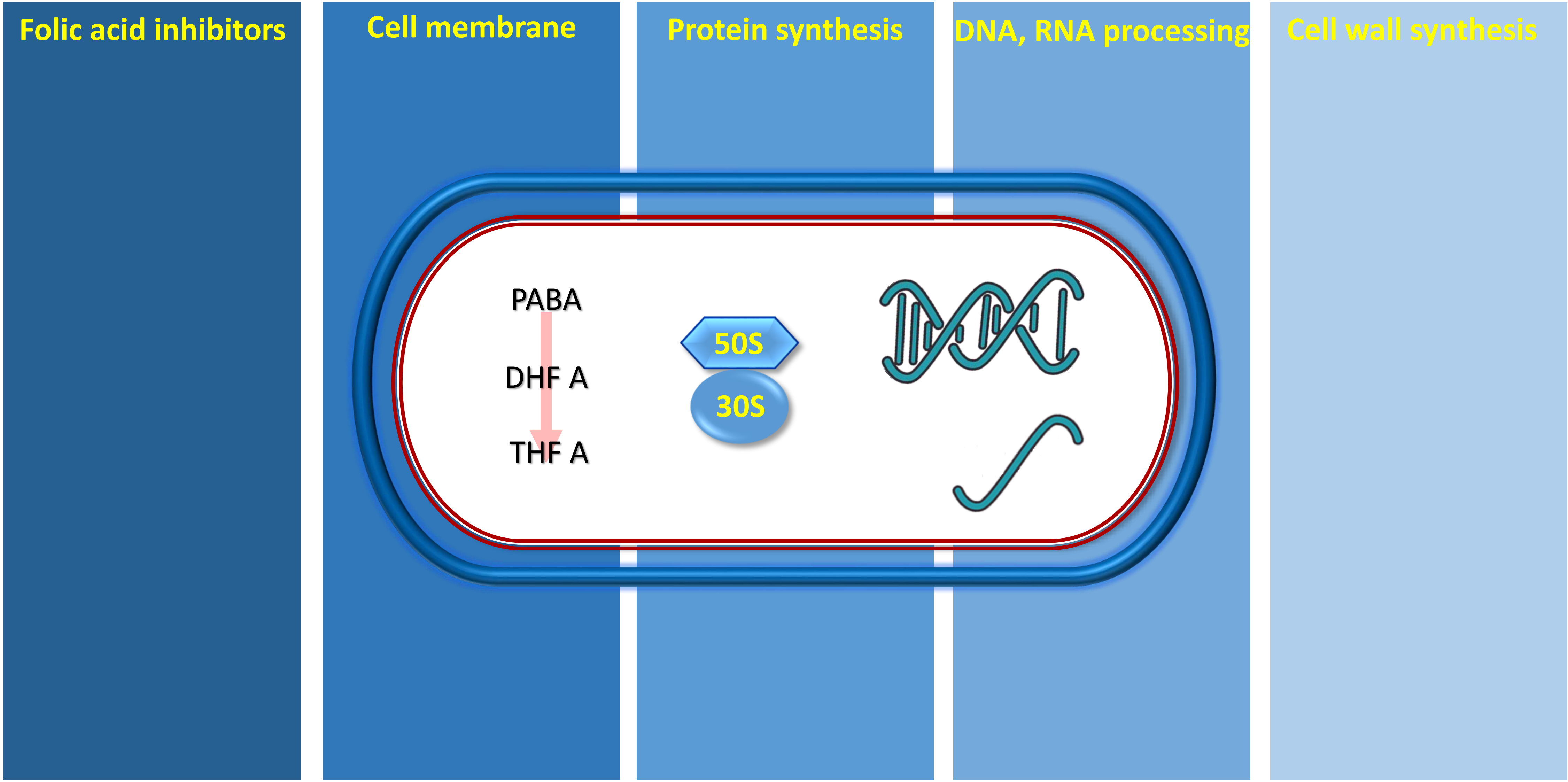
Quinolons

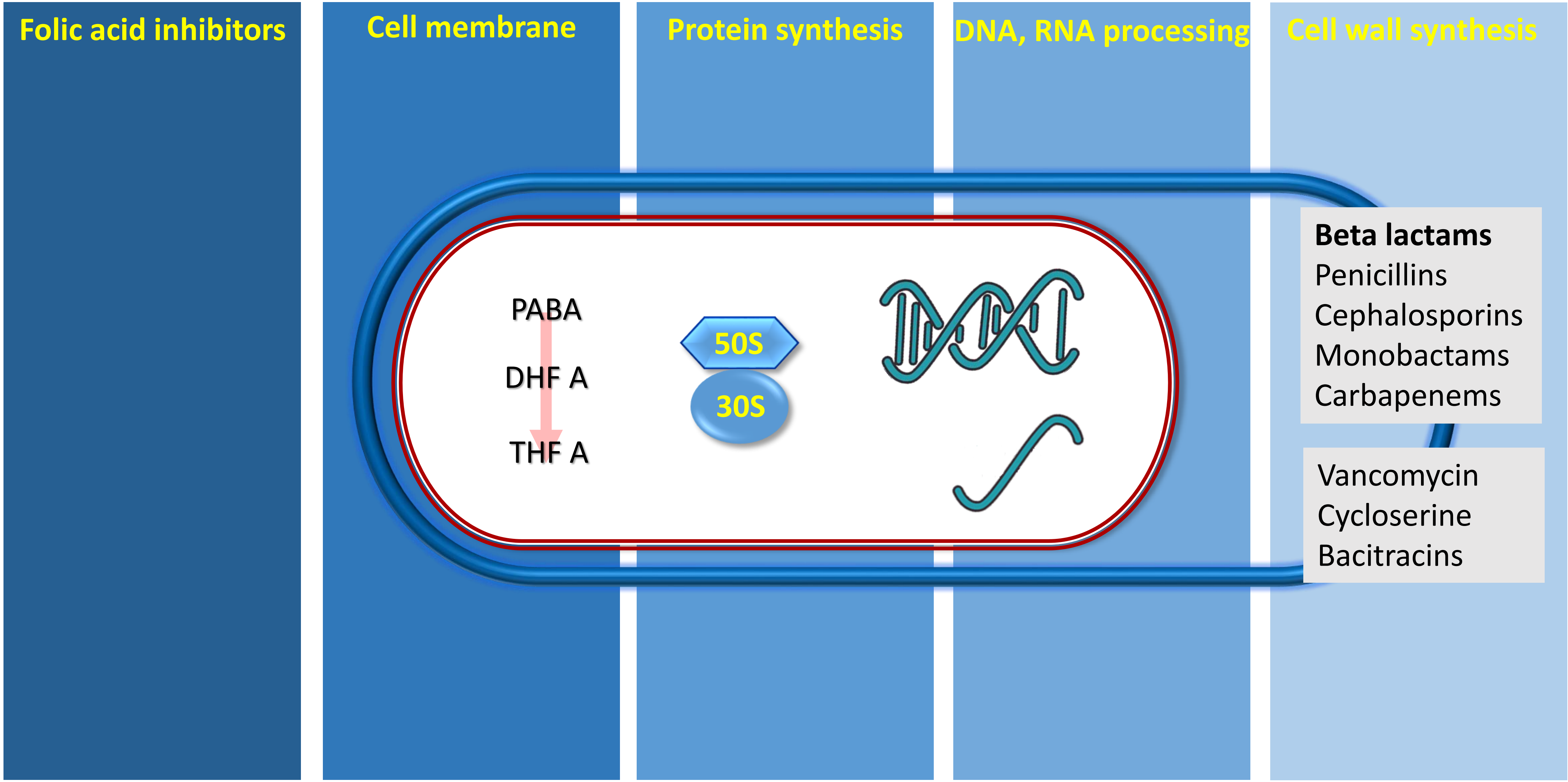
Carbapenems

Introduction and clinical use of antibiotics

Antimicrobial agents (mechanism)

1. Inhibition of the cell wall synthesis
2. Inhibition of the nucleic acid synthesis
3. Inhibition of the ribosome function
4. Inhibition of the cell membrane function
5. Inhibition of the folate metabolism


Folic acid inhibitors
Cell membrane
Protein synthesis
DNA, RNA processing
Cell wall synthesis
PABA
DHF A
THF A
50S
30S


Folic acid inhibitors
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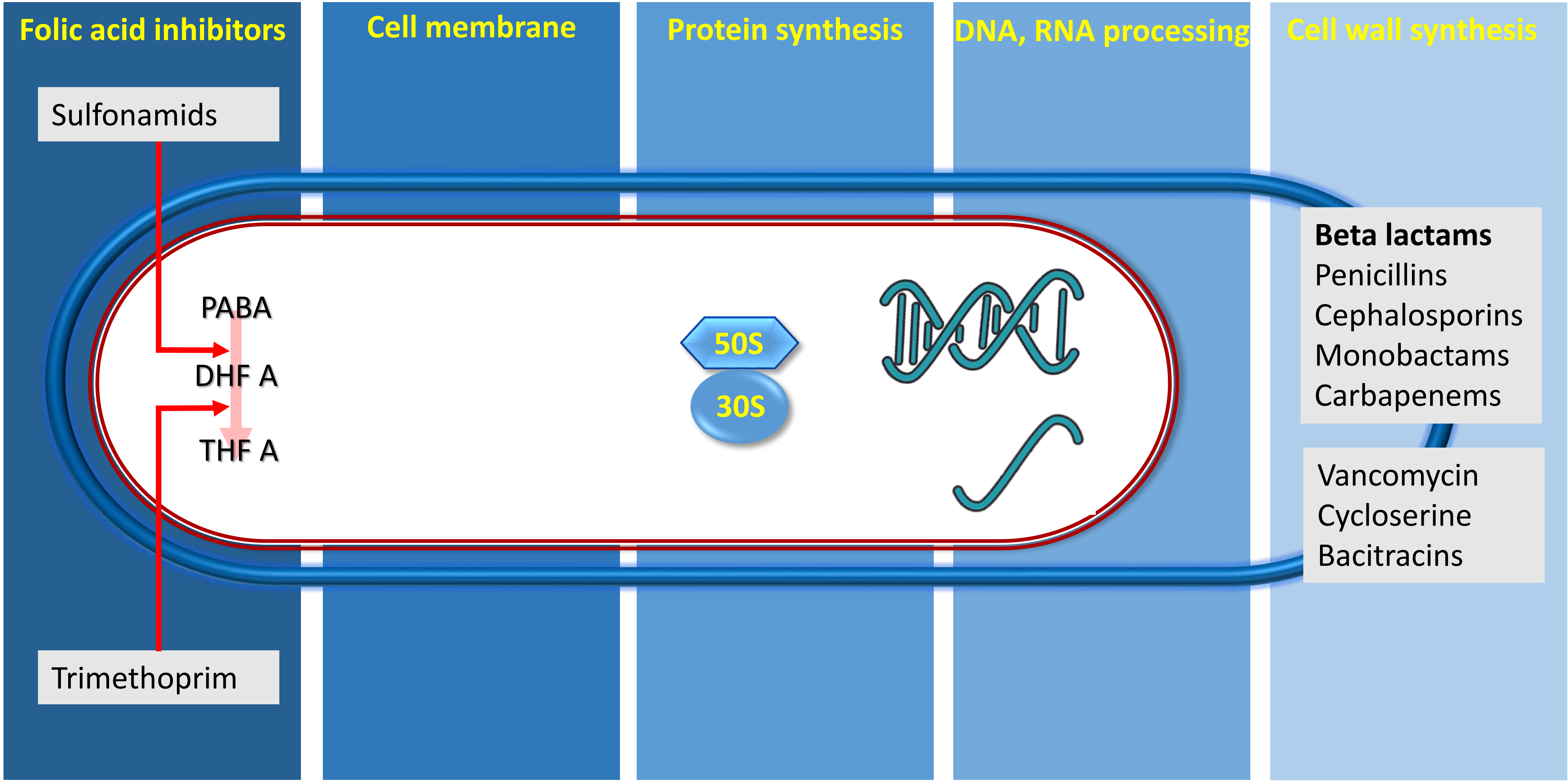
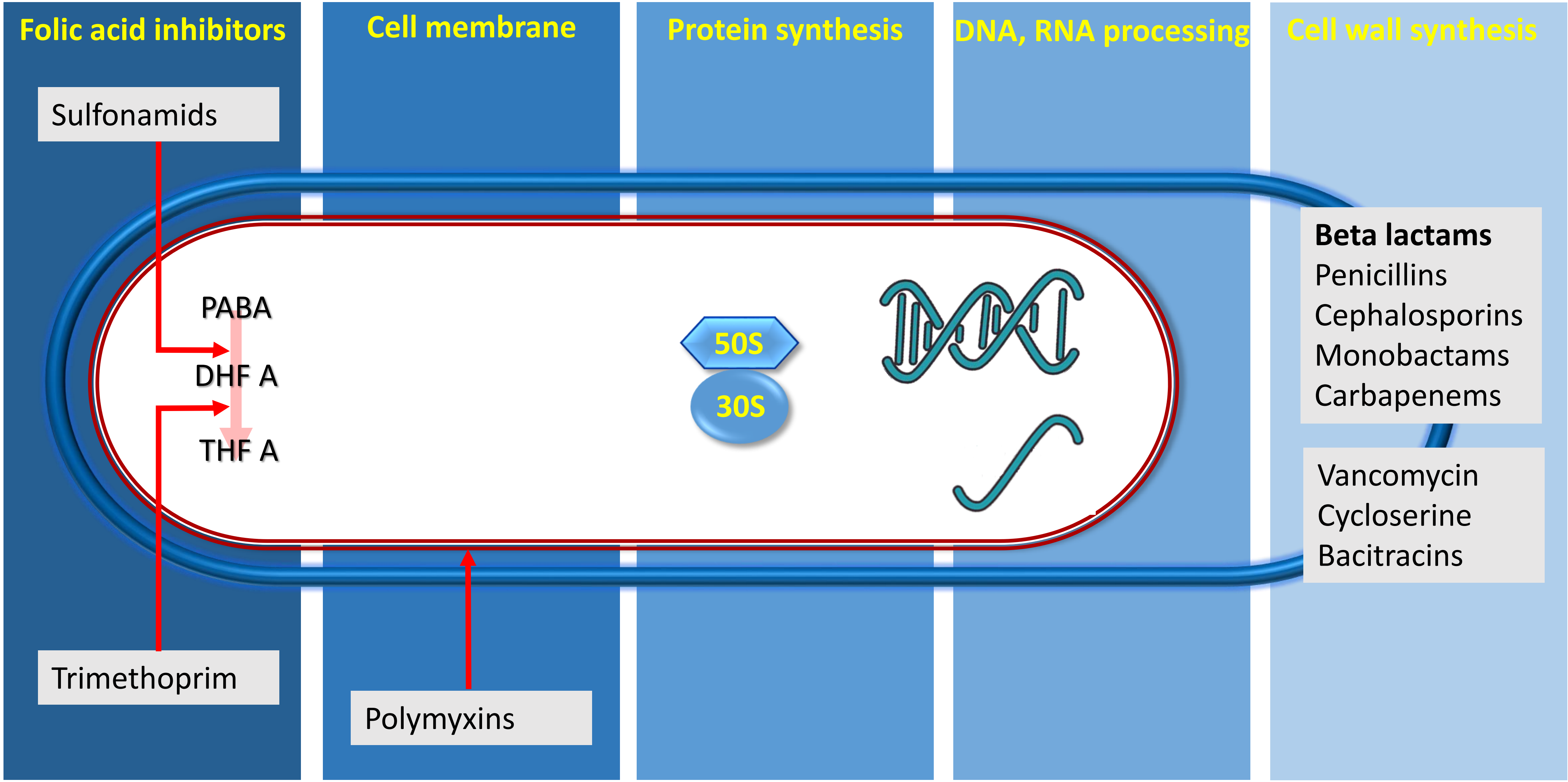
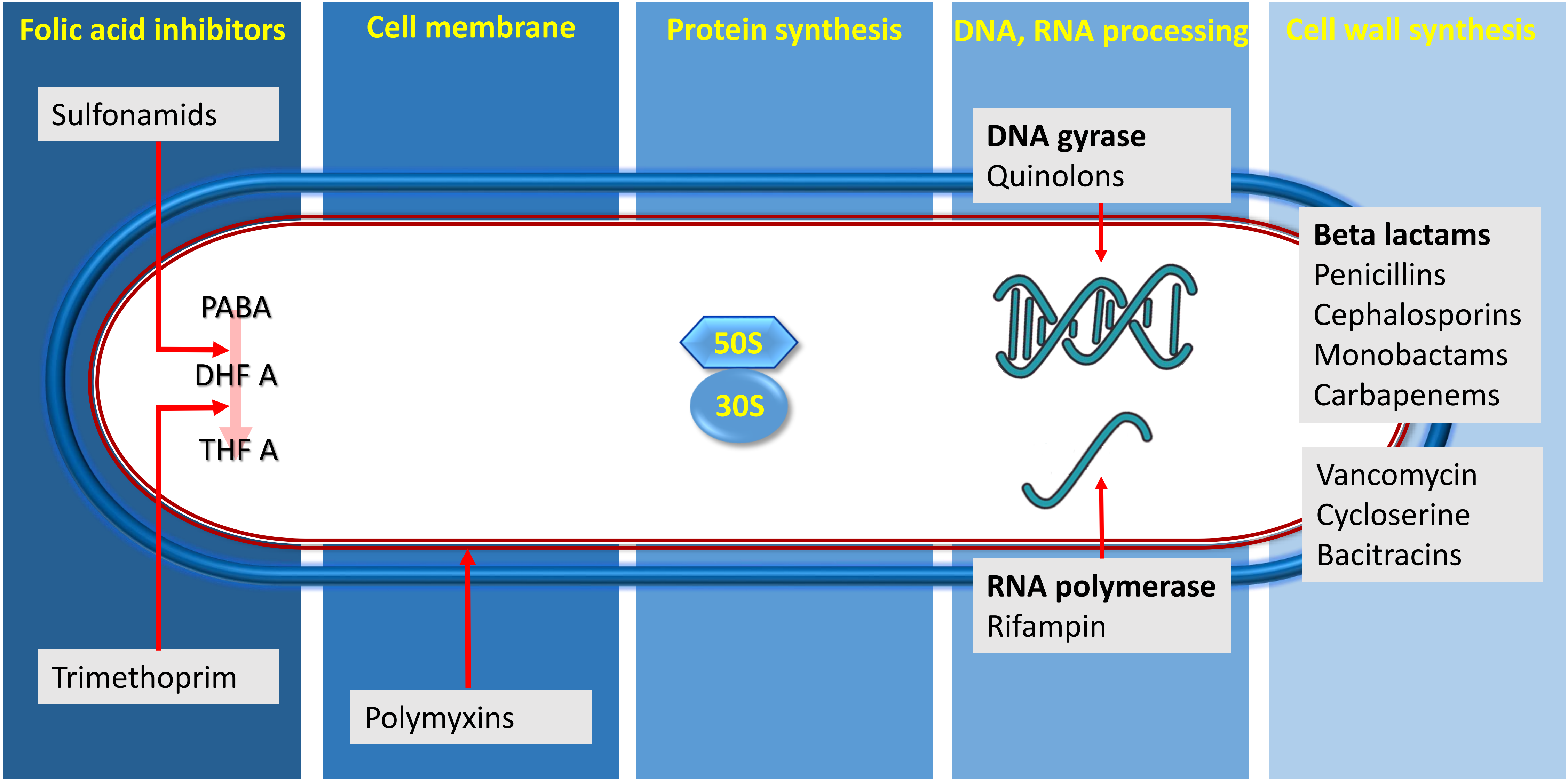
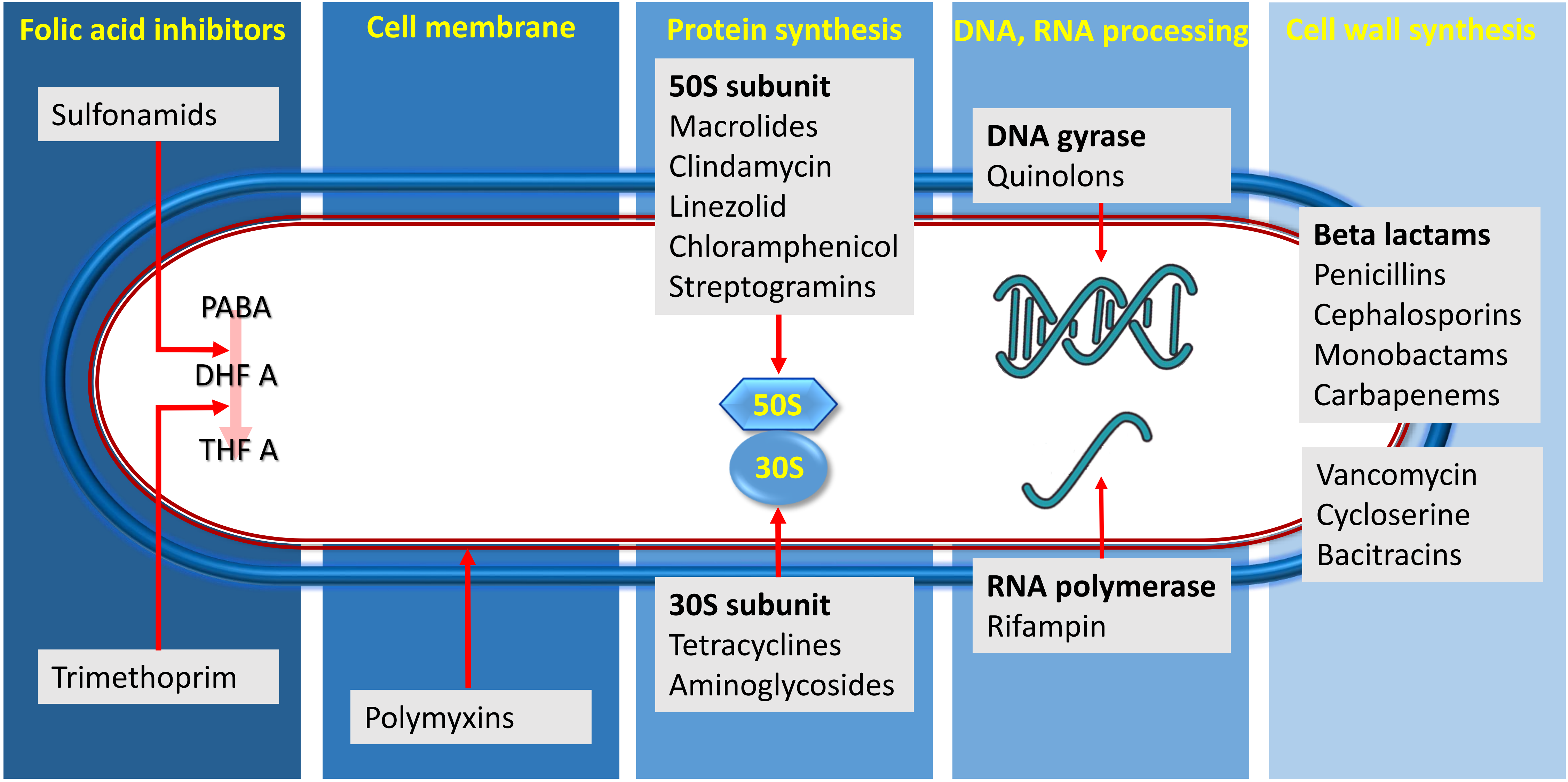
PABA
DHF A
THF A
50S
30S
Beta lactams
Penicillins
Cephalosporins
Monobactams
Carbapenems
Vancomycin
Cycloserine
Bacitracins

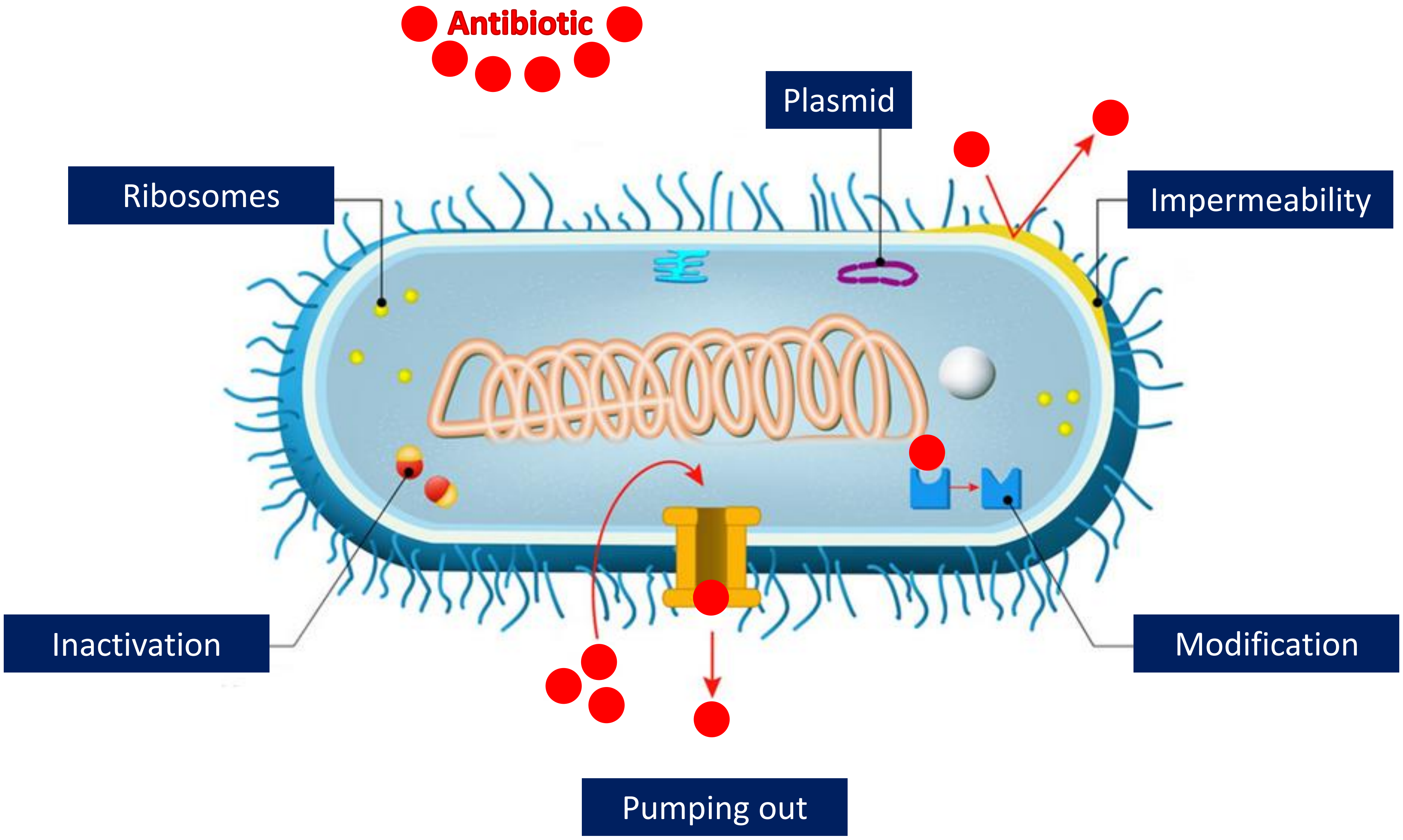

Folic acid inhibitors
Cell membrane
Protein synthesis
DNA, RNA processing
Cell wall synthesis
Sulfonamids
PABA
DHF A
THF A
Trimethoprim
50S
30S
Beta lactams
Penicillins
Cephalosporins
Monobactams
Carbapenems
Vancomycin
Cycloserine
Bacitracins


Folic acid inhibitors
Cell membrane
Protein synthesis
DNA, RNA processing
Cell wall synthesis
Sulfonamids
PABA
DHF A
THF A
Trimethoprim
Polymyxins
50S
30S
Beta lactams
Penicillins
Cephalosporins
Monobactams
Carbapenems
Vancomycin
Cycloserine
Bacitracins


Folic acid inhibitors
Cell membrane
Protein synthesis
DNA, RNA processing
Cell wall synthesis
Sulfonamids
PABA
DHF A
THF A
Trimethoprim
Polymyxins
50S
30S
DNA gyrase
Quinolons
RNA polymerase
Rifampin
Beta lactams
Penicillins
Cephalosporins
Monobactams
Carbapenems
Vancomycin
Cycloserine
Bacitracins


Folic acid inhibitors
Cell membrane
Protein synthesis
DNA, RNA processing
Cell wall synthesis
Sulfonamids
PABA
DHF A
THF A
Trimethoprim
Polymyxins
50S subunit
Macrolides
Clindamycin
Linezolid
Chloramphenicol
Streptogramins
50S
30S
30S subunit
Tetracyclines
Aminoglycosides
DNA gyrase
Quinolons
RNA polymerase
Rifampin
Beta lactams
Penicillins
Cephalosporins
Monobactams
Carbapenems
Vancomycin
Cycloserine
Bacitracins

Mechanism of action of AMR



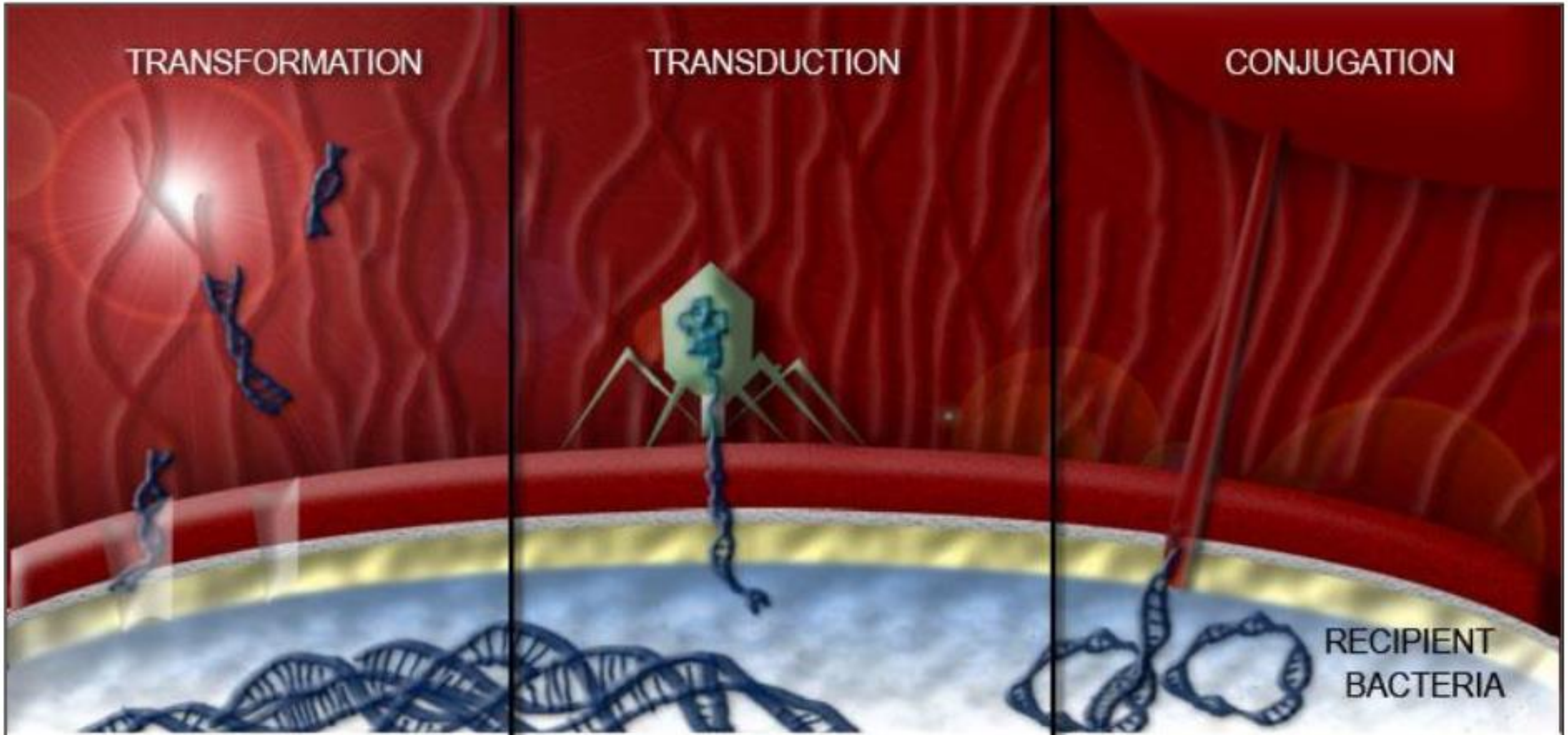
Resistance mechanism of individual antibiotics

Antibiotic class	Resistance type	Resistance mechanism	Common example
Aminoglycoside	Decreased uptake	Changes in outer membrane	*P. aeruginosa*
	Enzymatic modification	AGE's	Gram-negative bacteria
Beta-lactams	Altered PBP	PBP 2a	Mec A in *S. aureus*, CONS, *S. pneumoniae*
	Enzymatic degradation	Penicillinase which are classified as per ambler classification	Gram-negative bacteria
Glycopeptides	Altered target	D-alanyl-alanine is changed to D-alanyl-D-lactate	Vancomycin resistance in *E. faecium* and *E. faecalis*
Macrolides	Altered target	Methylation of ribosomal active site with reduced binding	*erm*-encoded methylases *in S. aureus*, *S. pneumoniae*, and *S. pyogenes*
	Efflux pumps	Mef type pump	*S. pneumoniae* and *S. pyogenes*
Oxazolidinones	Altered target	Mutation leading to reduced binding to active site	*E. faecium* and *S. aureus*
Quinolones	Altered target	Mutation leading to reduced binding to active site(s)	Mutations in gyr A in enteric Gram-negative bacteria and *S. aureus*
	Efflux	Membrane transporters	Mutations in gyr A and par C in *S. pneumoniae*. Nor-A in *S. aureus*
Tetracyclines	Efflux	New membrane transporters	*tet* genes encoding efflux proteins in Gram-positive and Gram-negative bacteria
	Altered target	Production of proteins that bind to the ribosome and alter the conformation of the active site	*tet* (M) and *tet* (O) in Gram-positive and Gram-negative bacteria species
Chloramphenicol	Antibiotic inactivation	Chloramphenicol acetyl transferase	CAT in *S. pneumonia*
	Efflux pump	New membrane transporters	cml A gene and flo gene efflux in *E. coli*
Sulfa drugs	Altered target	Mutation of genes encoding DHPS	*E. coli*, *S. aureus*, *S. pneumoniae*

DHPS=Dihydropteroate synthase, P. aeruginosa=Pseudomonas aeruginosa, S. aureus=Staphylococcus aureus, S. pneumoniae=Streptococcus pneumoniae, E. faecium=Enterococcus faecium, E. faecalis=Enterococcus faecalis, S. pyogenes=Streptococcus pyogenes, E. coli=Escherichia coli, PBP=Penicillin binding protein, AGE's=Aminoglycoside modifying enzymes, CAT=Chloramphinecol acetyl transferases

GENETIC BASIS of AMR

- Mutation in genes
  - Modifications of the antimicrobial target
  - A decrease in the drug uptake
  - Activation of efflux mechanisms to extrude the harmful molecule
  - Global changes in important metabolic pathways
- Acquisition of foreign DNA (HGT)
  - Transformation (incorporation of naked DNA)
  - Transduction (phage mediated)
  - Conjunction (bacterial Sex)

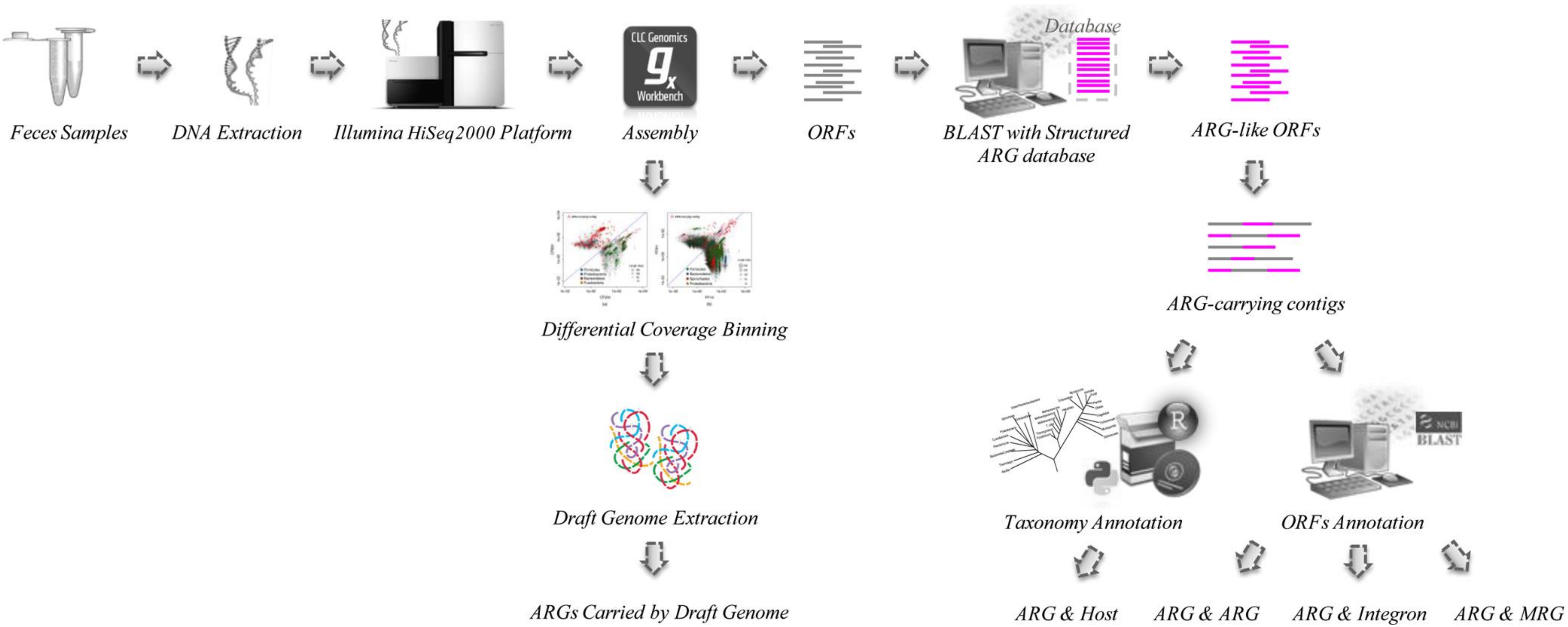


Transformation involves uptake of short fragments of naked DNA by naturally transformable bacteria.

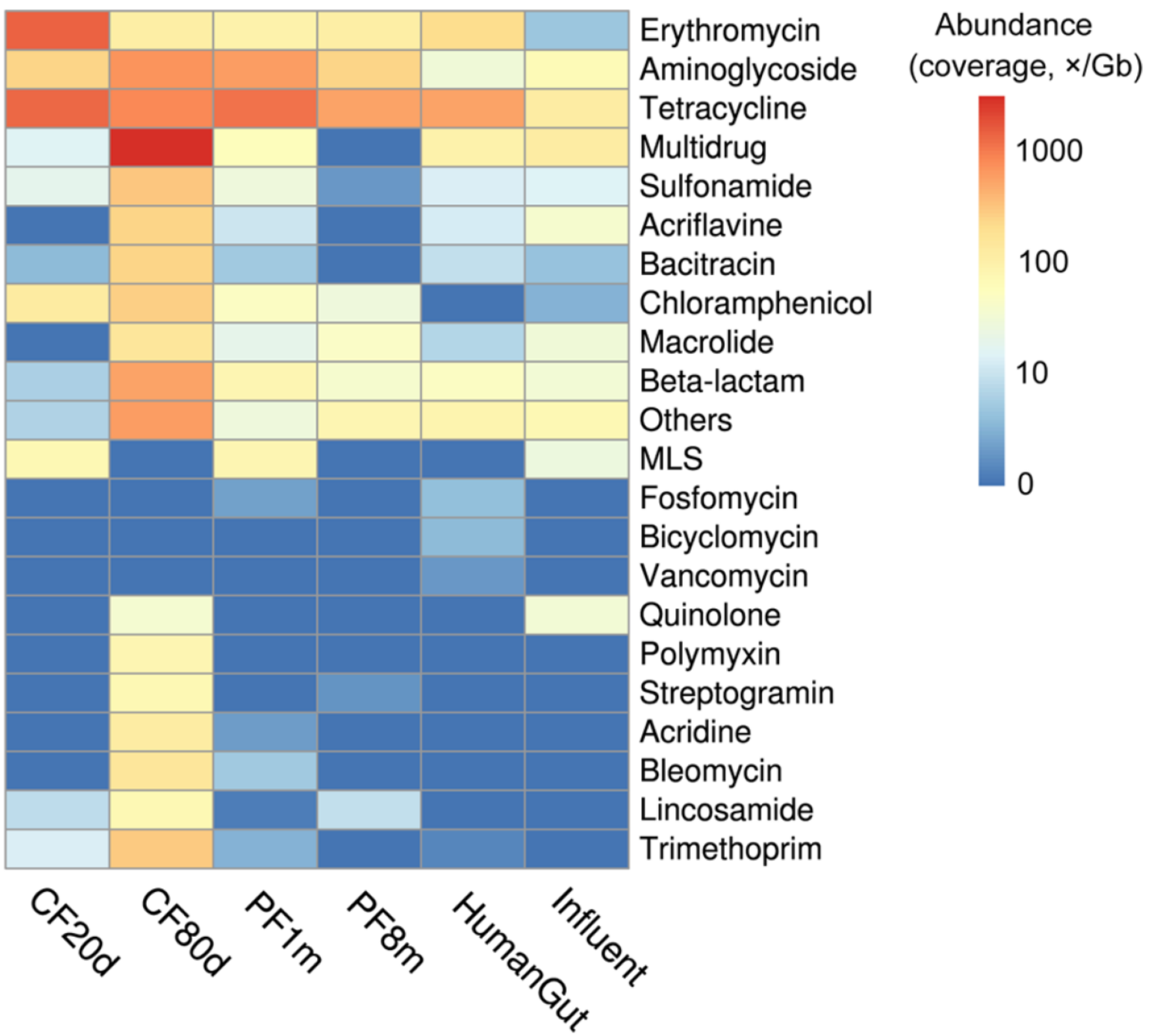
Transduction involves transfer of DNA from one bacterium into another via bacteriophages

Conjugation involves transfer of DNA material via sexual pilus and requires cell-to-cell contact

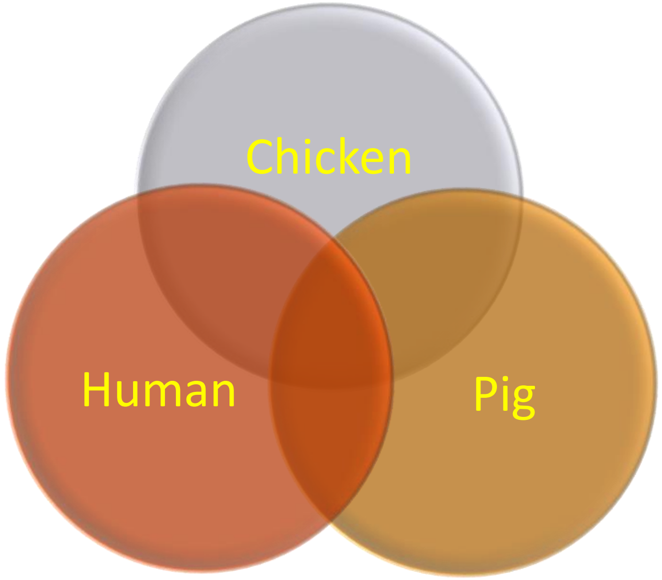
Metagenomic Assembly Reveals Hosts of Antibiotic Resistance Genes and the Shared Resistome in Pig, Chicken, and Human Feces



Liping etal, 2016



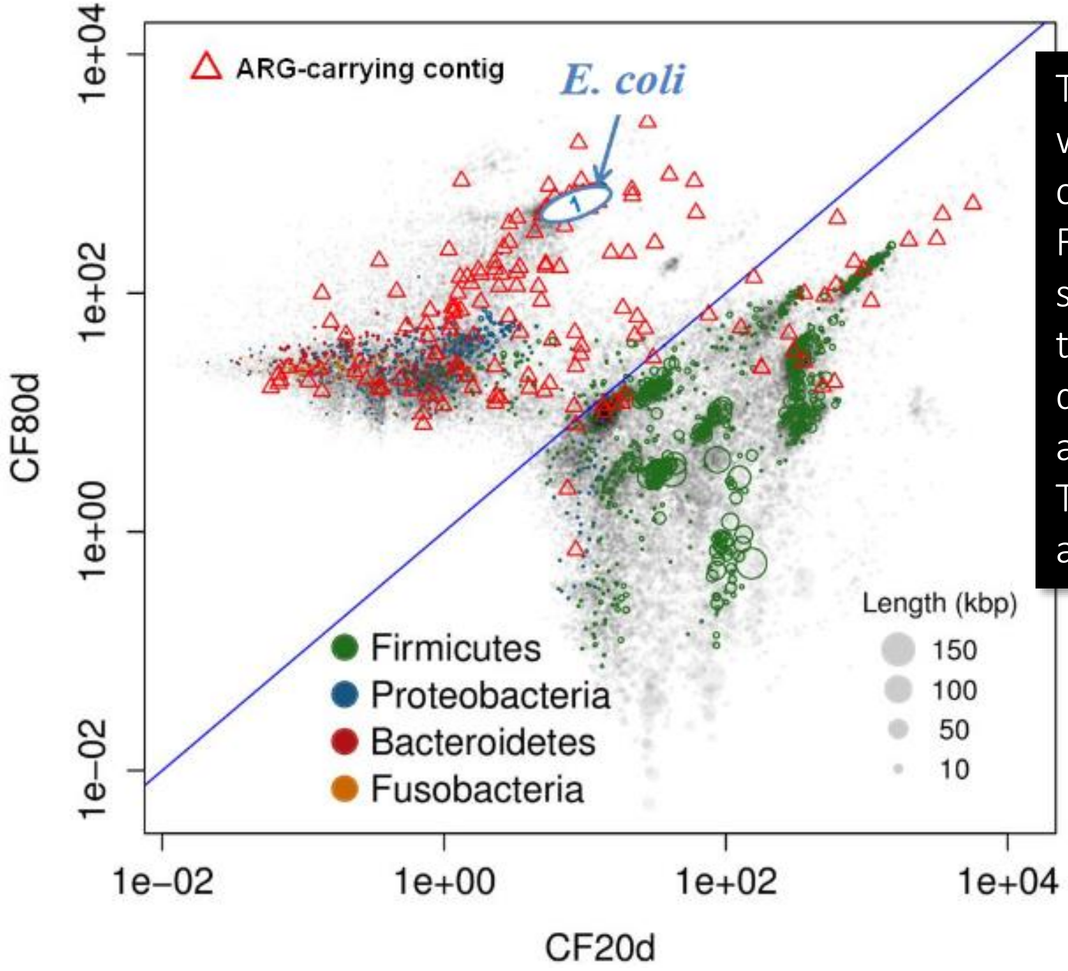
Erythromycin
Aminoglycoside
Tetracycline
Multidrug
Sulfonamide
Acriflavine
Bacitracin
Chloramphenicol
Macrolide
Beta-lactam
Others
MLS
Fosfomycin
Bicyclomycin
Vancomycin
Quinolone
Polymyxin
Streptogramin
Acridine
Bleomycin
Lincosamide
Trimethoprim
CF20d
CF80d
PF1m
PF8m
HumanGut
Influent
Abundance
(coverage, ×/Gb)
1000
100
10
0



A comparison of metagenomes from feces of chickens, pigs and humans showed a high homology to **tetracycline genes** (tetA) and the presence of gene combinations of individual resistance elements, which encode for resistance to **beta-lactams**, **aminoglycosides**, **macrolides** and multidrug.

Liping etal, 2016

Distribution of ARG-carrying contigs in chicken metagenome data sets via differential coverage-based binning. All nodes represent scaffolds, scaled by the square root of their length, and colored by community taxonomy.



The abundance of this E. coli bin was increased 77-fold in CF80d compared to CF20d.
Functional analysis of this E. coli strain using MEGAN KEGG revealed that the strain might cause human disease and could possess antibiotic and metal resistance. Therefore, this E. coli strain is likely a high-risk human pathogen.

Liping etal, 2016


DO NOT
ENTER


HOSPITAL

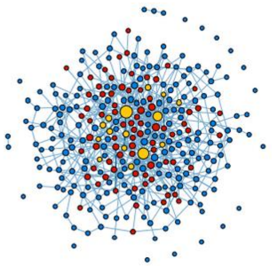
How does resistance spread and develops?

Poultry to human

Antibiotics used on livestock

Socio-demographic

Occupational risk



Antibiotics used on companion animals

Antibiotics pollute the environment

Import of livestock and food

Travel

Human to human

Use

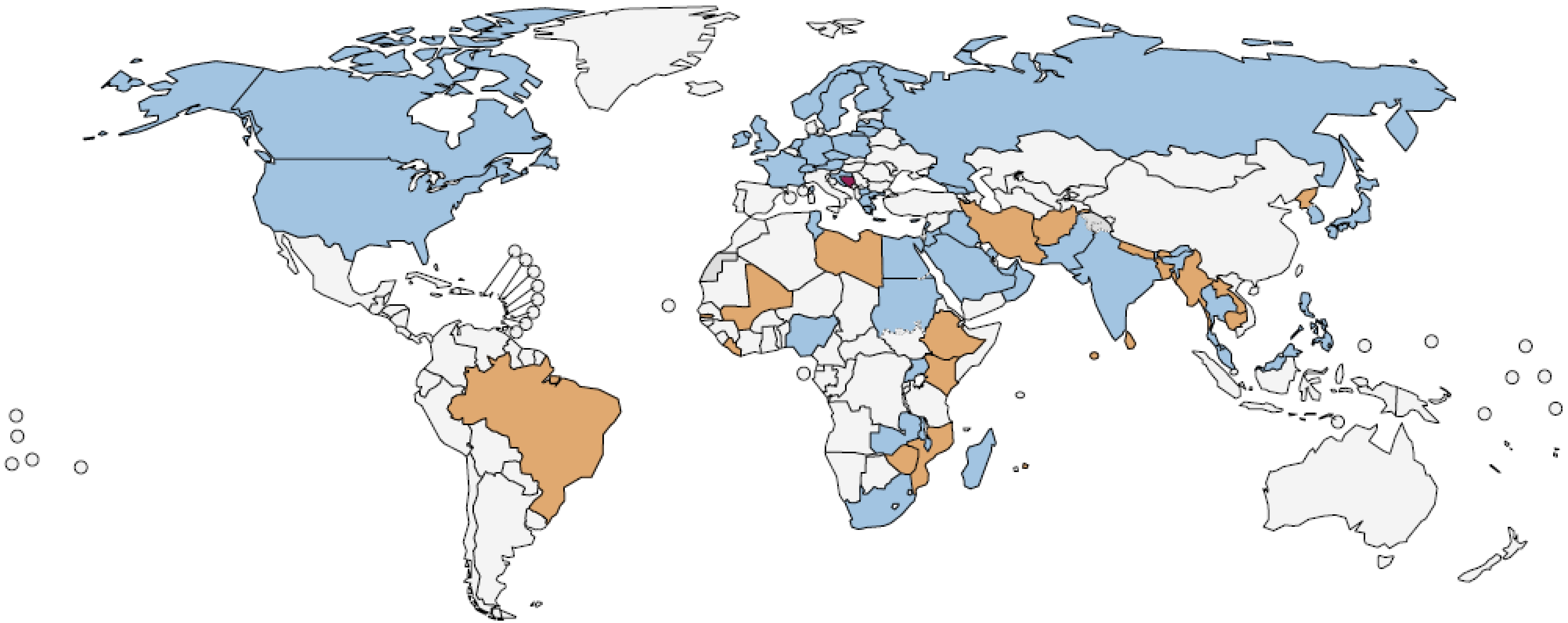
Overuse

Abuse

Misuse

World Health Organization


Countries enrolled in GLASS (n=71)
Data not available
Not applicable



Countries reporting information on the implementation of their national surveillance system
Countries reporting AMR rates and information on the implementation of their national surveillance system
Countries reporting AMR rates
Data not available
Not applicable

Germany

Population 82.11 million

The national surveillance of AMR is coordinated by the Robert Koch Institute, offering a publically accessible interactive database for data of the AMR surveillance system (Antibiotika Resistenz Surveillance – ARS). The National action plan on prevention of AMR (DART 2020) was published in 2015. Germany participates in the EARS-NET and has been enrolled in GLASS since September 2016.

Current status of the national AMR surveillance system

18752 surveillance sites
623 hospitals
18129 outpatient facilities

53 laboratories performing AST
EQA provided to all labs for bacterial identification and AST for some GLASS pathogen

Surveillance Sites

Surveillance Sites

Surveillance Sites

NRL
selected
AST standard
CLSI, EUCAST
EQA
provided

National Reference Laboratory

National Coordinating Centre

NCC
established
National AMR surveillance plan
in place (not defined budget)
National Focal Point
appointed

GLASS

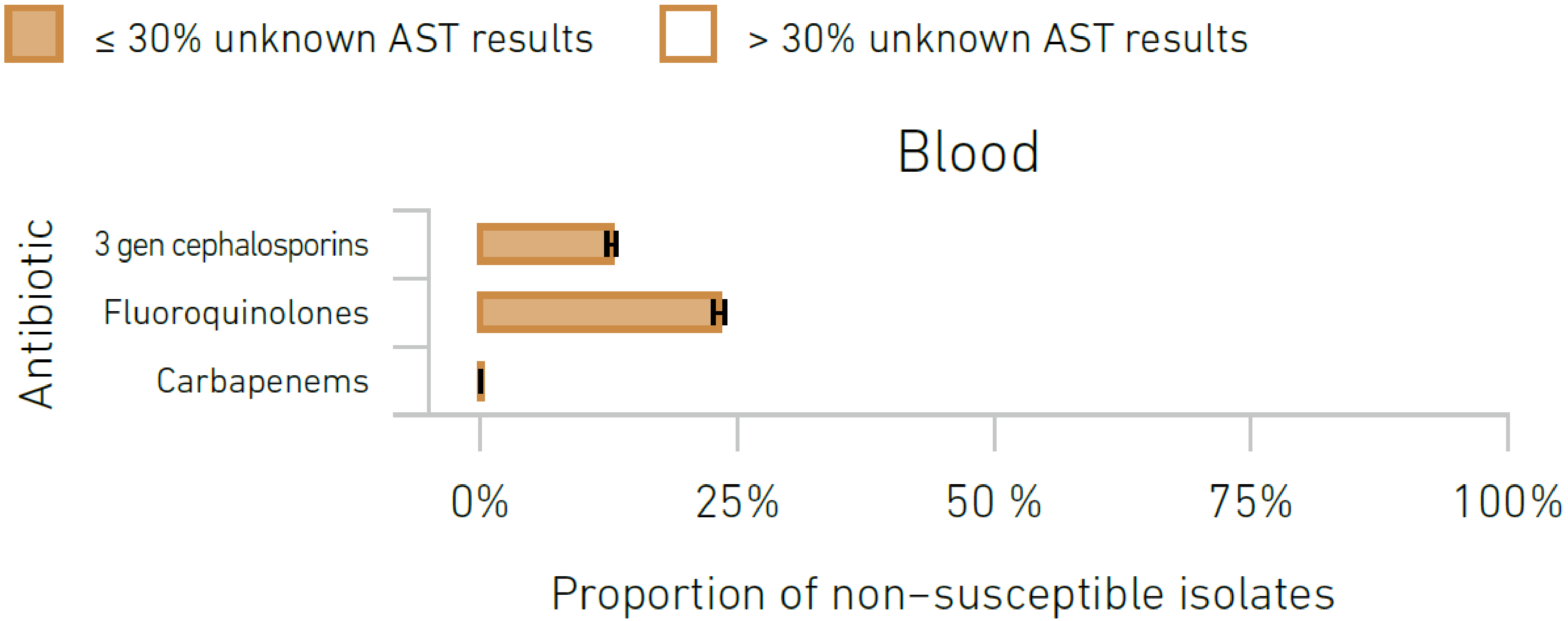
in 2018 data call
479 surveillance sites providing data to GLASS
(479 hospitals)

Data submission

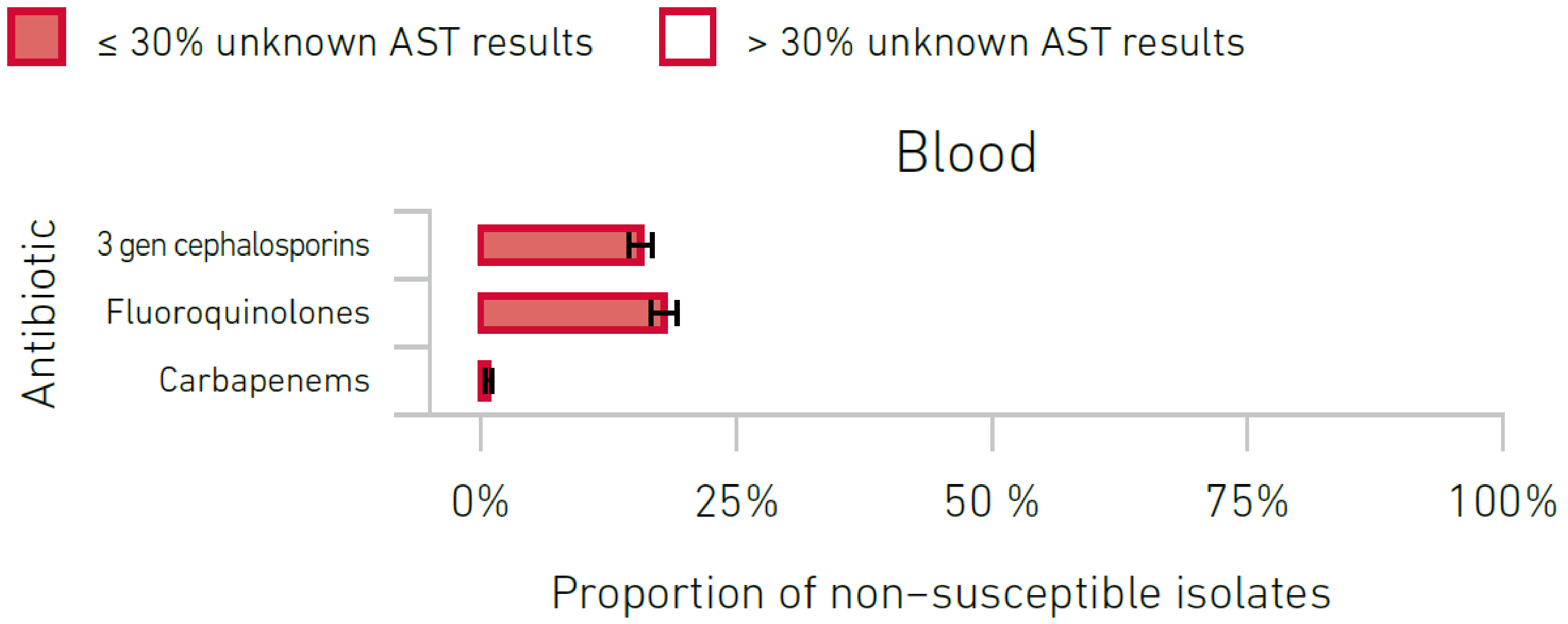
Specimen type	Data on number of tested patient	Pathogen	AST results	Age	Gender	Infection origin
Blood	<70% data collected	*Acinetobacter* spp.	100% data collected	100% data collected	99-70% data collected	<70% data collected
		E. coli	100% data collected	100% data collected	99-70% data collected	<70% data collected
		K. pneumoniae	100% data collected	100% data collected	99-70% data collected	<70% data collected
		Salmonella spp.	100% data collected	100% data collected	100% data collected	<70% data collected
		S. aureus	100% data collected	100% data collected	99-70% data collected	<70% data collected
		S. pneumoniae	100% data collected	100% data collected	99-70% data collected	<70% data collected
Urine	<70% data collected	*E. coli*	<70% data collected	<70% data collected	99-70% data collected	<70% data collected
		K. pneumoniae	<70% data collected	<70% data collected	<70% data collected	<70% data collected
Stool	<70% data collected	*Salmonella* spp.	<70% data collected	<70% data collected	<70% data collected	<70% data collected
		Shigella spp.	<70% data collected	<70% data collected	<70% data collected	<70% data collected
Genital	<70% data collected	*N. gonorrhoeae*	<70% data collected	<70% data collected	<70% data collected	<70% data collected

100% data collected   99-70% data collected   <70% data collected

Escherichia coli



Klebsiella pneumoniae



Iran (Islamic Republic of)

Population 81.16 million

Iran has developed its National Action Plan on AMR with promotion and development of AMR surveillance included in the NAP.
Iran has been enrolled in GLASS since May 2016.

Current status of the national AMR surveillance system

12 surveillance sites
6 hospitals
6 outpatient facilities

6 laboratories performing AST
EQA provided to all labs for bacterial identification and AST for all GLASS pathogens

Surveillance Sites

Surveillance Sites

Surveillance Sites

NRL selected
AST standard CLSI,
EQA provided

National Reference Laboratory

National Coordinating Centre

NCC established
National AMR surveillance plan in place (not defined budget)
National Focal Point appointed

GLASS
Global AMR Surveillance System

in 2018 data call
6 surveillance sites providing data to GLASS
(6 hospitals)

Data submission

Specimen type	Data on number of tested patient	Pathogen	AST results	Age	Gender	Infection origin
Blood	<70%	*Acinetobacter* spp.	100%	100%	100%	<70%
		E. coli	100%	100%	100%	<70%
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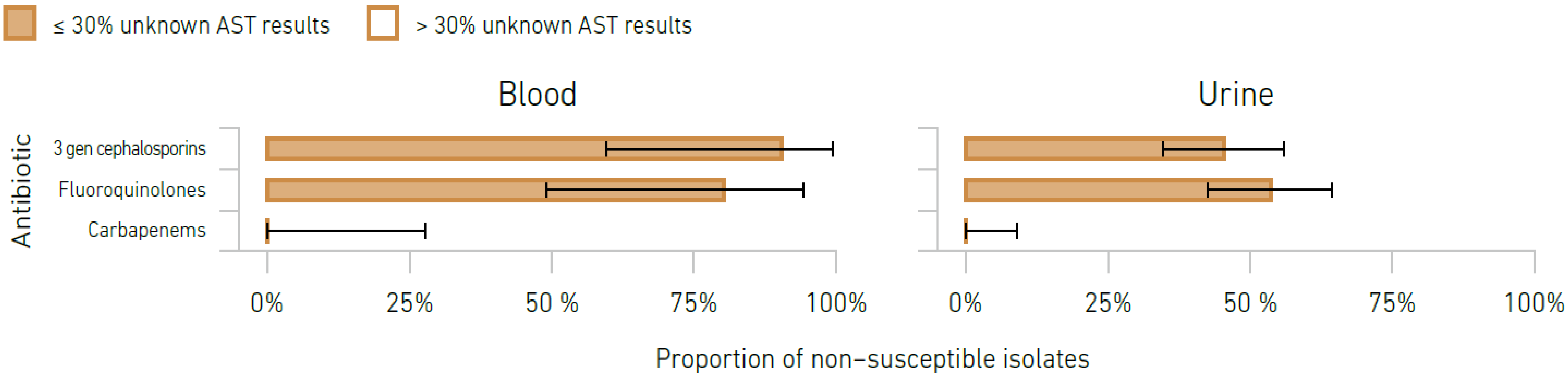
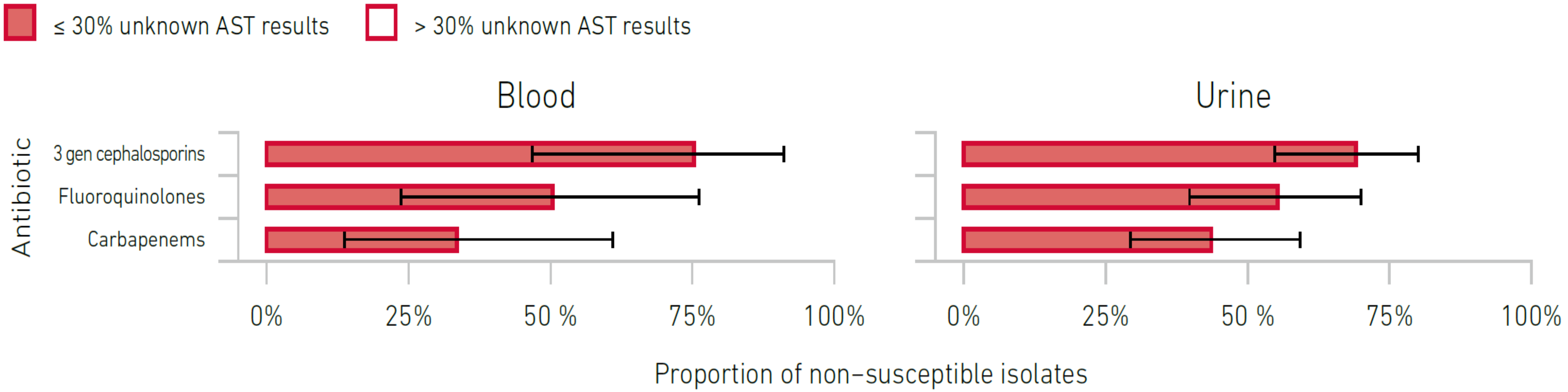
		Salmonella spp.	<70%	<70%	<70%	<70%
		S. aureus	<70%	<70%	<70%	<70%
		S. pneumoniae	<70%	<70%	<70%	<70%
Urine	<70%	*E. coli*	100%	100%	100%	<70%
		K. pneumoniae	100%	100%	100%	<70%
Stool	<70%	*Salmonella* spp.	<70%	<70%	<70%	<70%
		Shigella spp.	<70%	<70%	<70%	<70%
Genital	<70%	*N. gonorrhoeae*	<70%	<70%	<70%	<70%

100% data collected · 99-70% data collected · <70% data collected

Escherichia coli



Klebsiella pneumoniae



Antibiotic use, resistance and the link to nutrition

21st century

Antibiotic resistance as one of the three most important public health

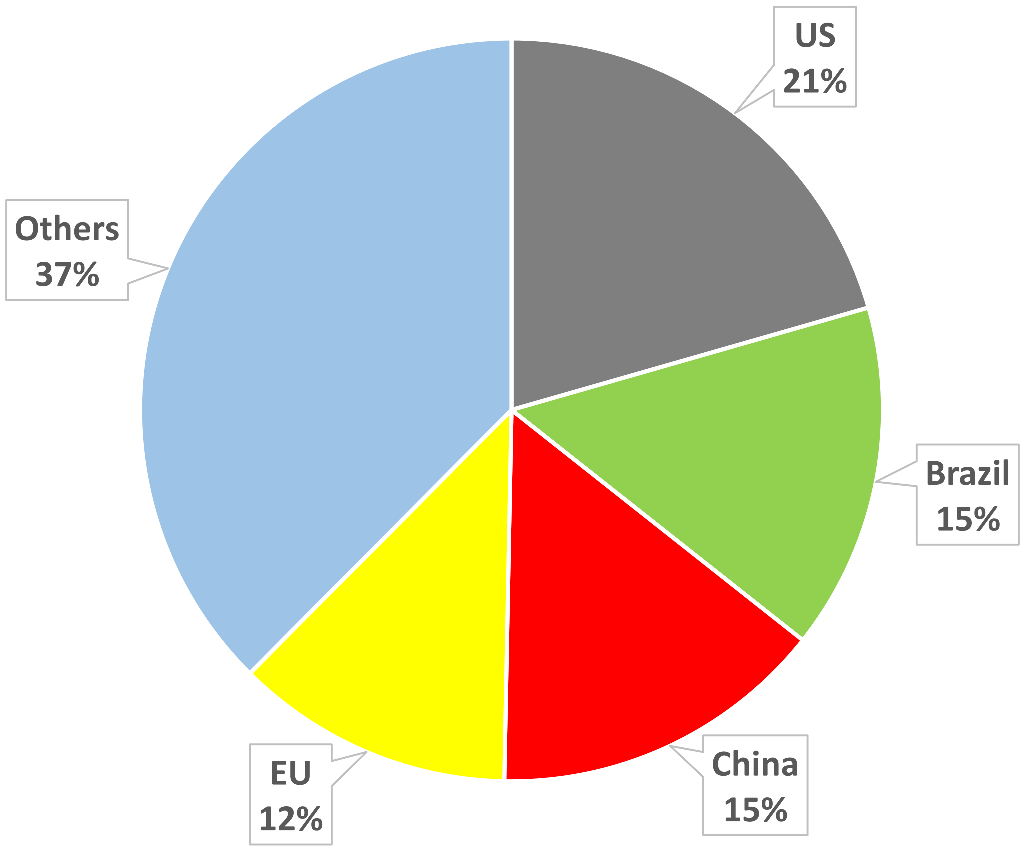
- According to a recent report, antibiotic resistance is estimated to cause around **300 million** premature deaths by 2050.
- with a loss of up to **$100 trillion** to the global economy.
- This situation is worsened by a paucity of a robust antibiotic pipeline, resulting in the emergence of infections that are almost untreatable and leaving clinicians with **no reliable alternatives** to treat infected patients.

Antimicrobial-resistant, from farm to fork

Antibiotic applications in poultry production

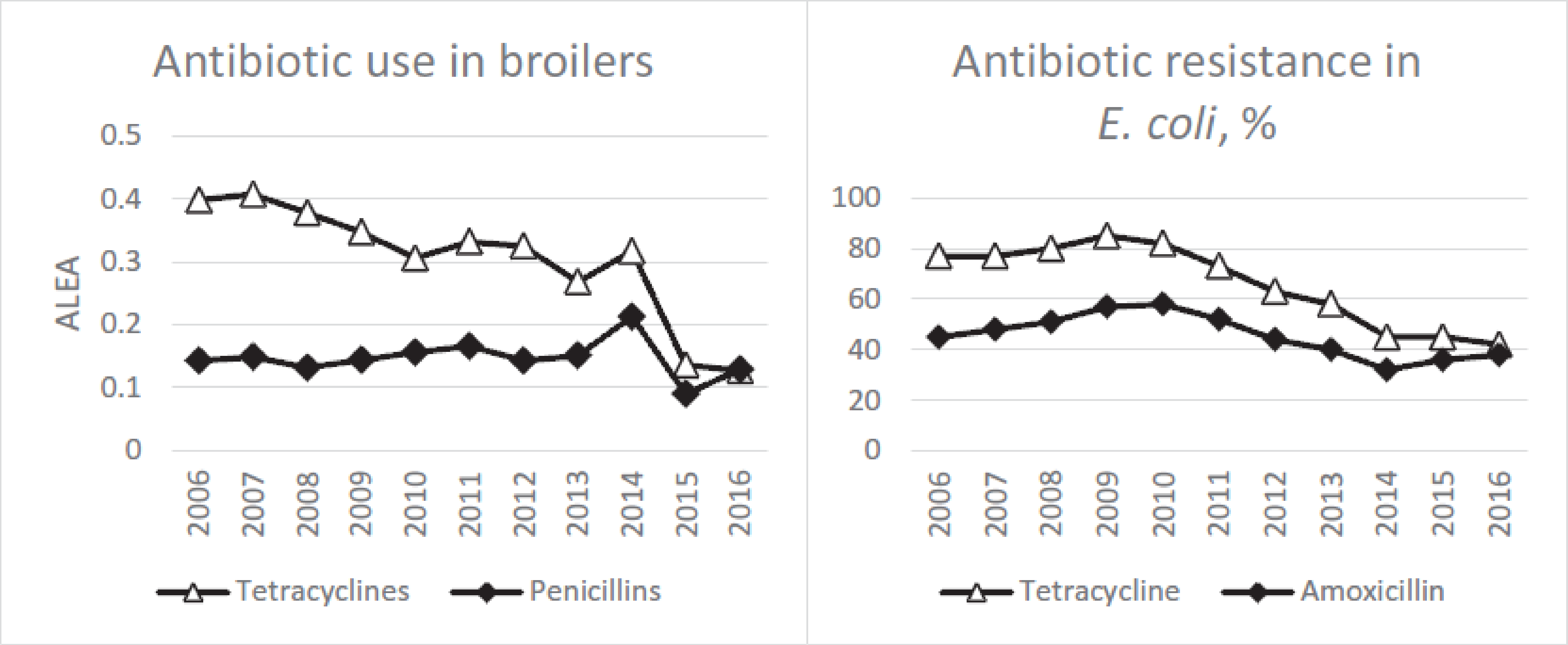
1. Therapeutics
2. Prophylaxis
3. Growth promotion

63% of the total worldwide broiler production

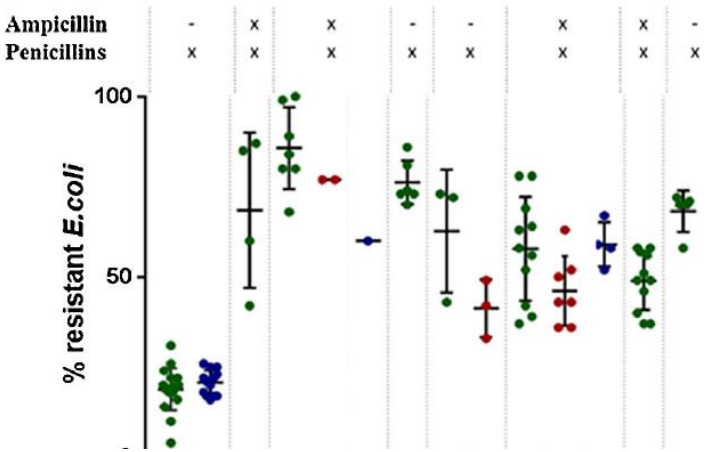


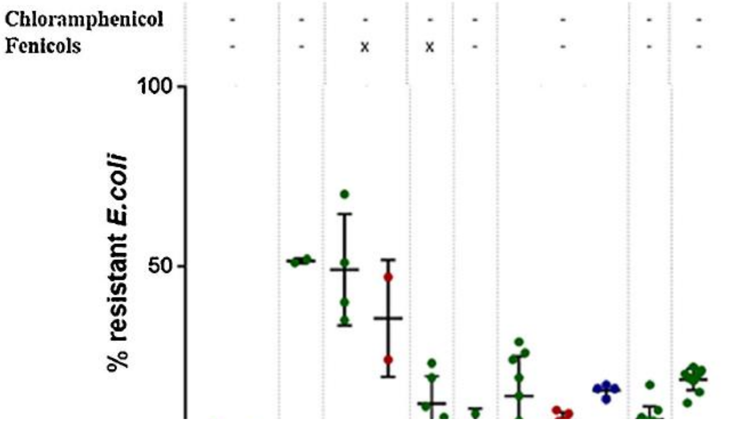
Roth et al. 2019, Poult. Sci.

Escherichia coli are commensal bacteria that are ubiquitous in animals and humans. Because of their widespread availability, monitoring of commensal bacteria allows the comparison of the selective pressure effects in all relevant populations and is considered useful as an early alert system, for tracking emerging resistance in livestock and possible spread to animal-derived food (EFSA, 2008). Due to this prevalence, they are widely accepted as indicator bacteria for AR in Gram-negative bacteria populations and serve as a model for studying the emergence of AR (Kaesbohrer et al., 2012).

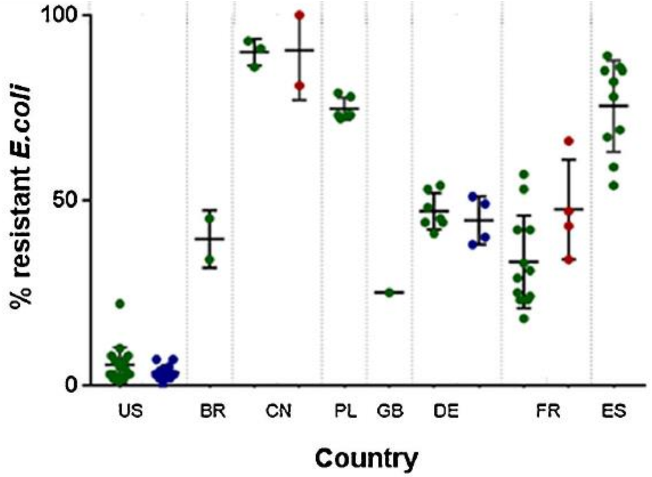


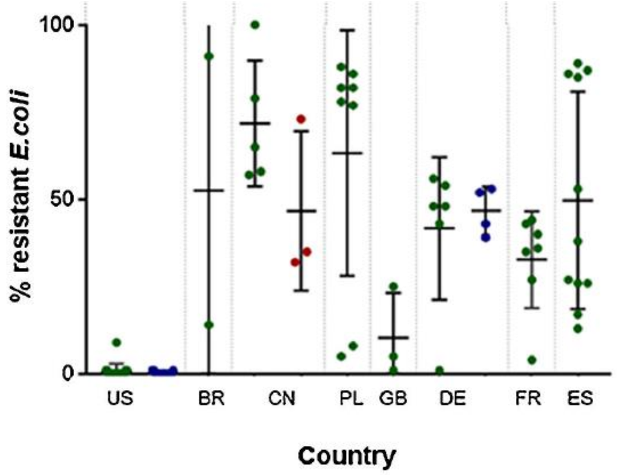
Antibiotic use in broilers
0.5
0.4
0.3
0.2
0.1
0
ALEA
2006
2007
2008
2009
2010
2011
2012
2013
2014
2015
2016
Tetracyclines
Penicillins
Antibiotic resistance in
E. coli, %
100
80
60
40
20
0
2006
2007
2008
2009
2010
2011
2012
2013
2014
2015
2016
Tetracycline
Amoxicillin





- ❑ The average resistance rates in *E. coli* to representatives of these antibiotic classes are higher than **40%** in all countries





Staphylococcus species

- Staphylococcosis
- pododermatitis (bumblefoot)
- septicaemia

first line of drugs → β-lactams

Germany reported → MRSA
- 77% in broilers
- 54% in Turkeys

It is worth noting that most of these organisms showed a high level of resistance to oxacillin and tetracycline, which would be disastrous if these oxacillin-resistant strains are transferred to humans

Salmonella species

- salmonellosis

first line of drugs → furazolidone, gentamycin sulfate, (other sulfa drugs)

Brazil reported → AMR of *SE*

- Sulfonamides 72.4
- florfenicol 59.2%

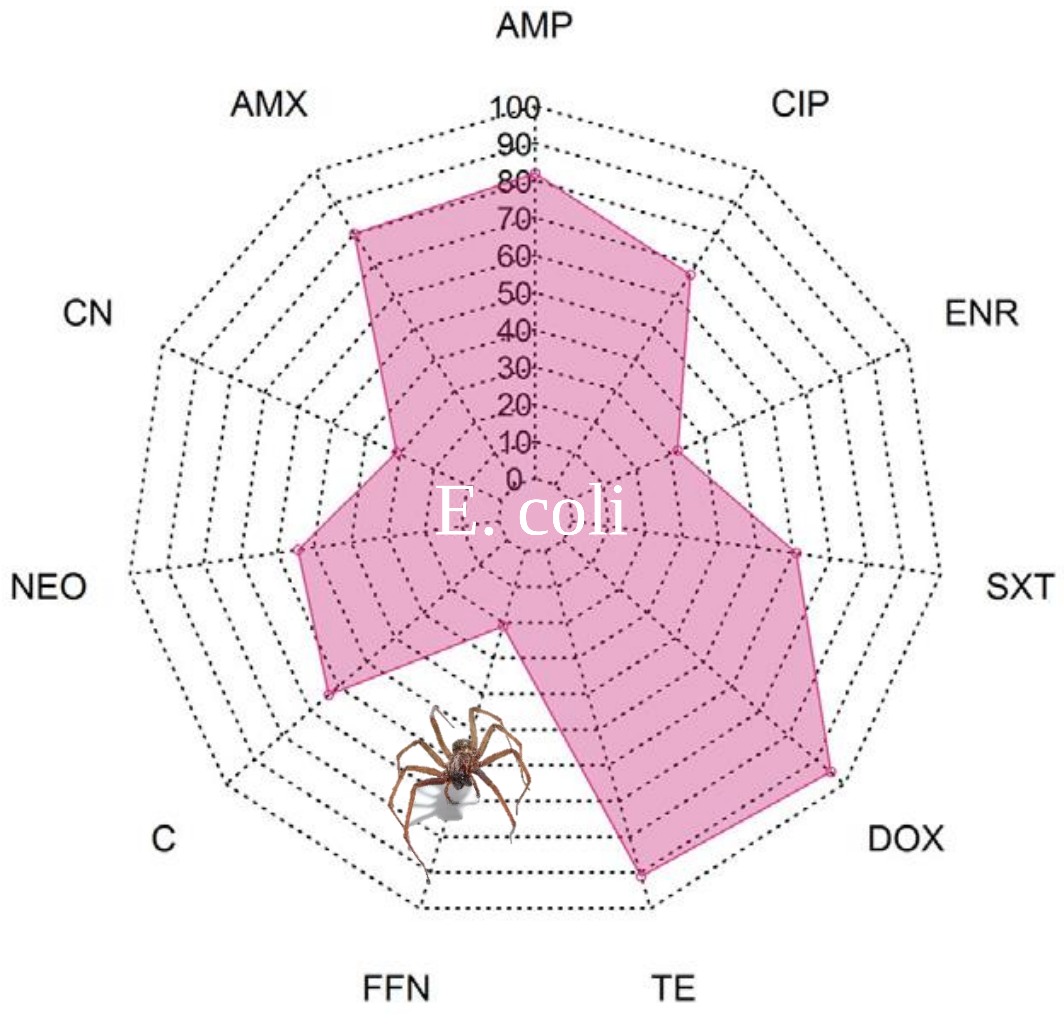
70 Published Articles

2000 - 2016

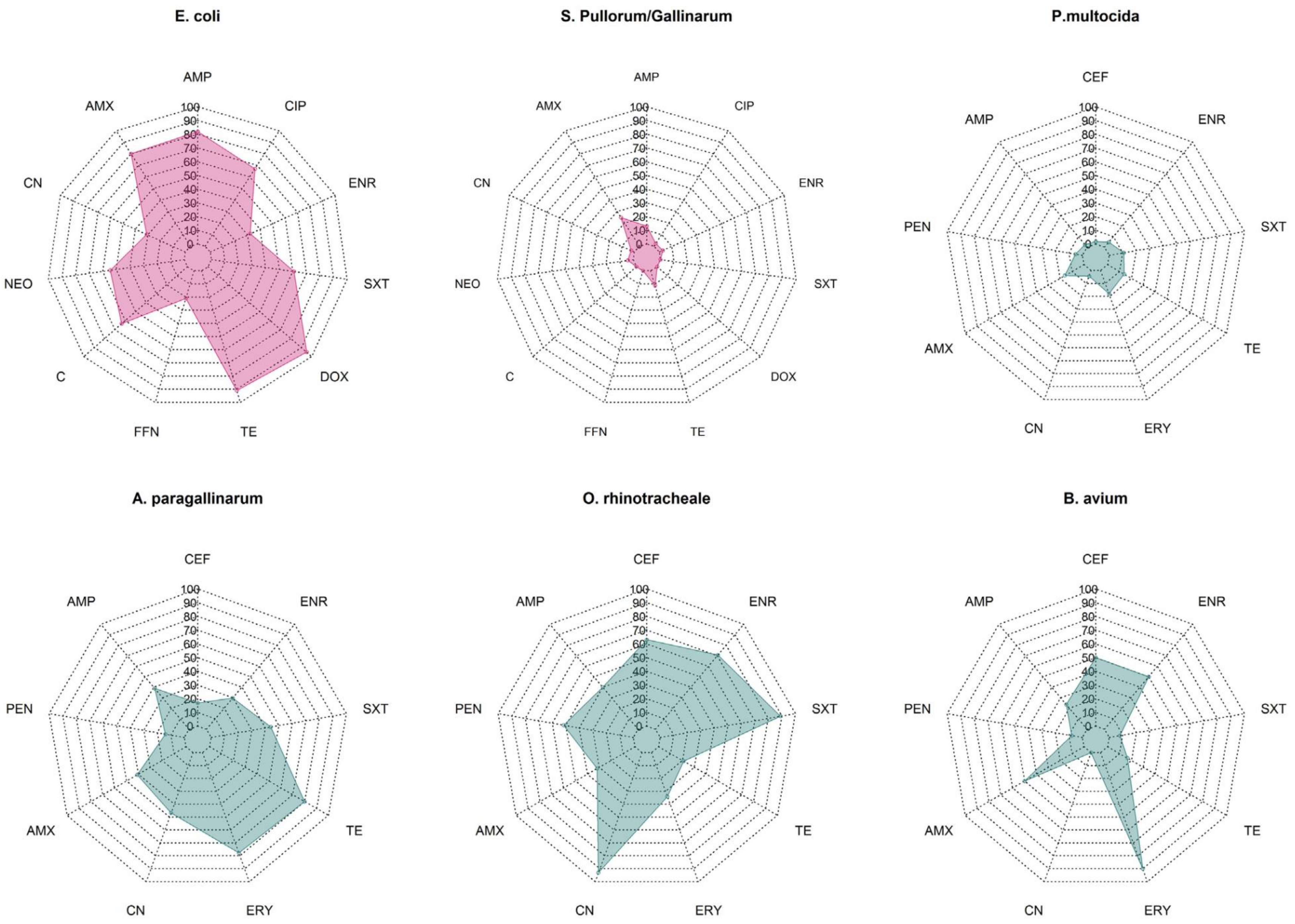
China, US, Canada,Jordan, Iran, Egypt, Brazil, Spain, Thailand, Korea, India, Zimbabwe, Hungary, Indonesia, Taiwan, Mexico Uganda, Peru, Ecuador, Panama, Belgium, Netherland, Germany, Sweden, Denmark and Norway

“E. coli,” “S. pullorum,” “S. gallinarum,” “Pasteurella multocida,” “Avibacterium paragallinarum,” “Haemophilus para-gallinarum,” “Mannheimia haemolitica,” “Gallibacterium anatis,” “Ornithobacterium rhinotracheale,” “Mycoplasma,” “Chlamydia psittaci,” “Bordetella avium,” “Riemerella anatipestifer,” “Pseudomonas aeruginosa,” “Mycobacterium avium,” “Clostridium perfringens,” and *“Erysipelothrix rhusiophathiae.”*

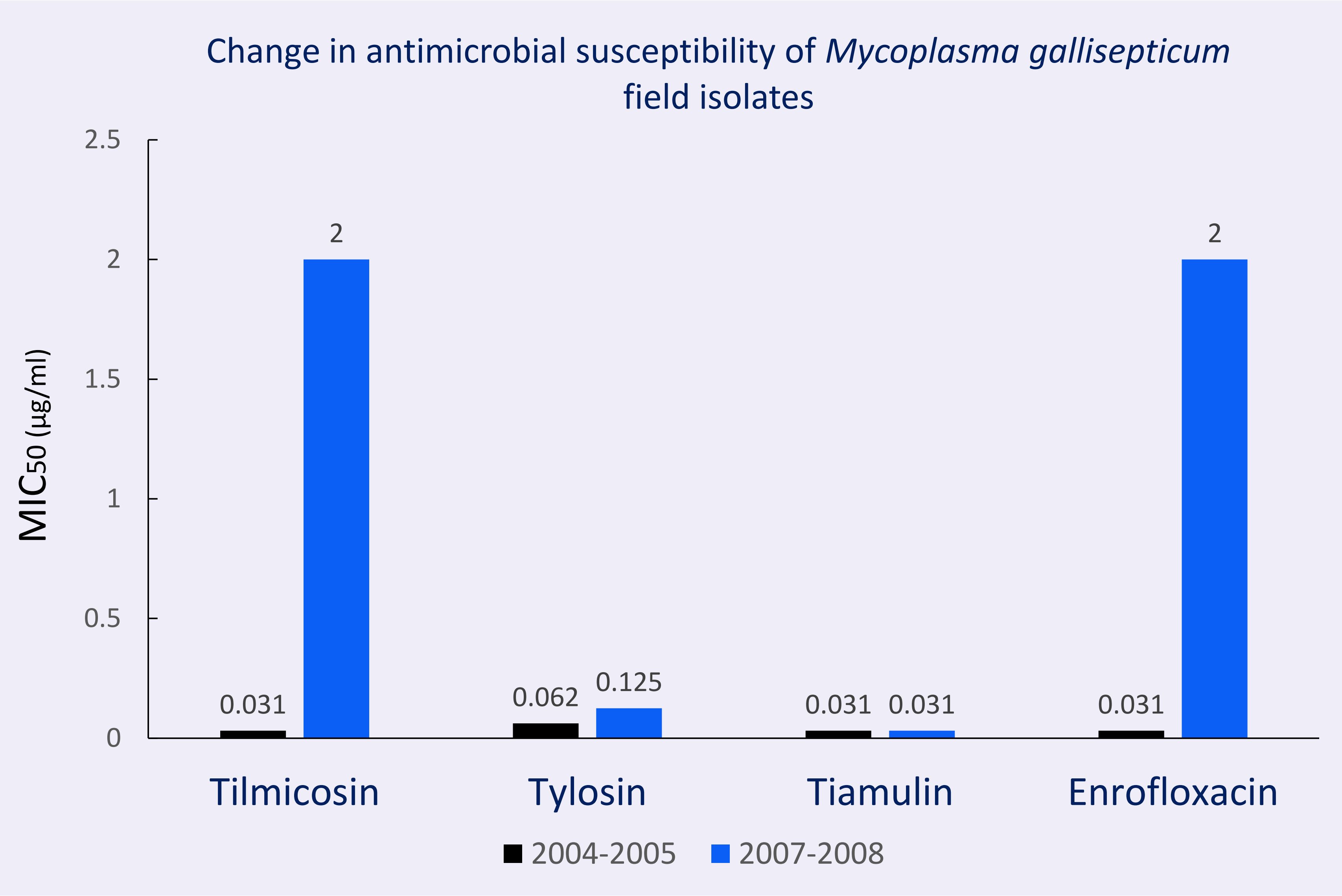
Nhung et al, 2017

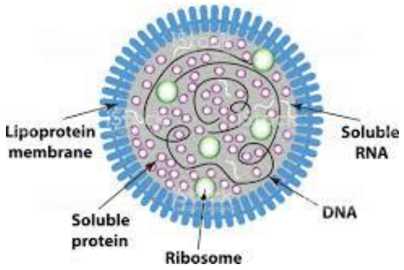


Nhung et al, 2017



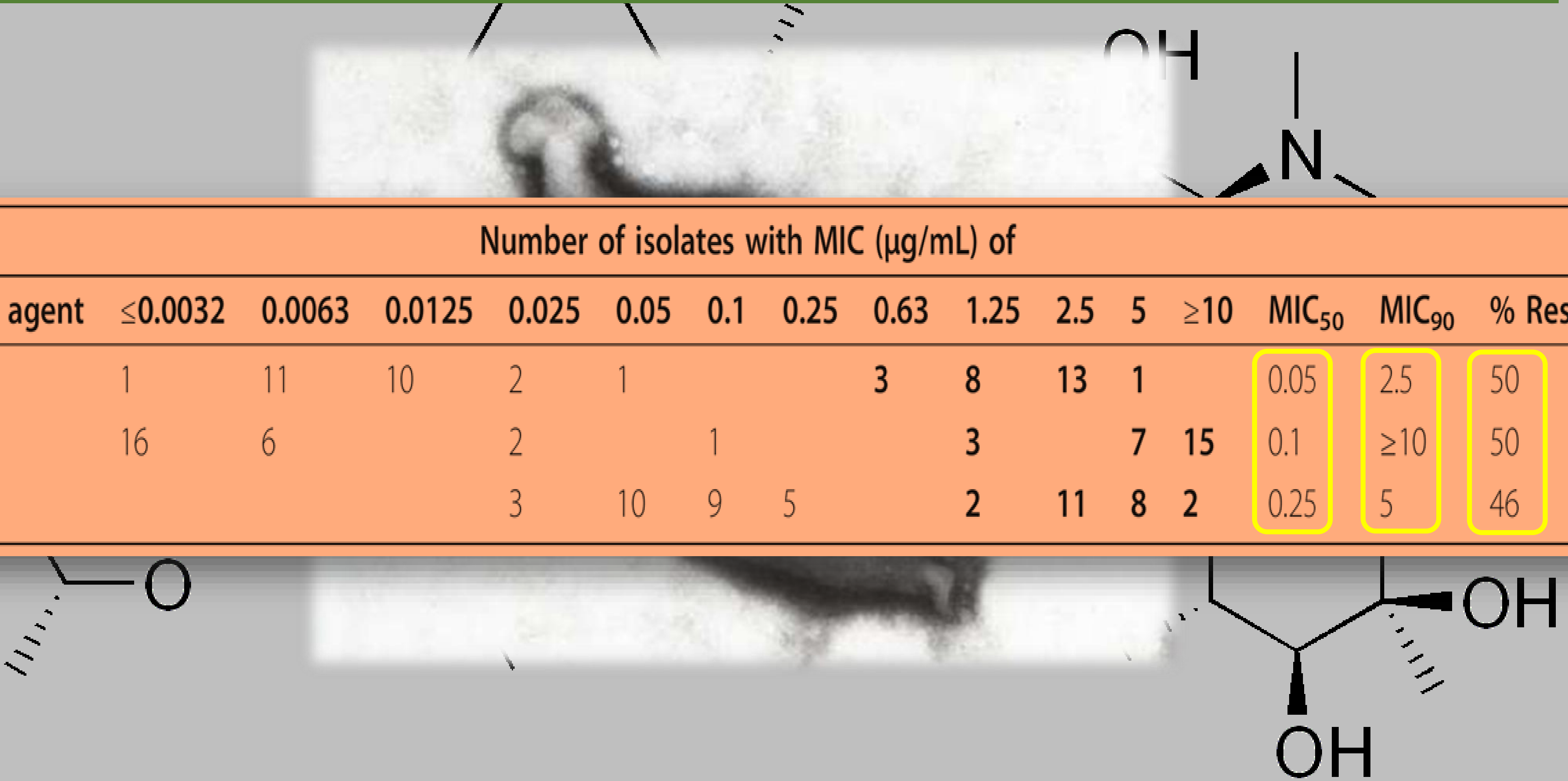
Nhung et al, 2017





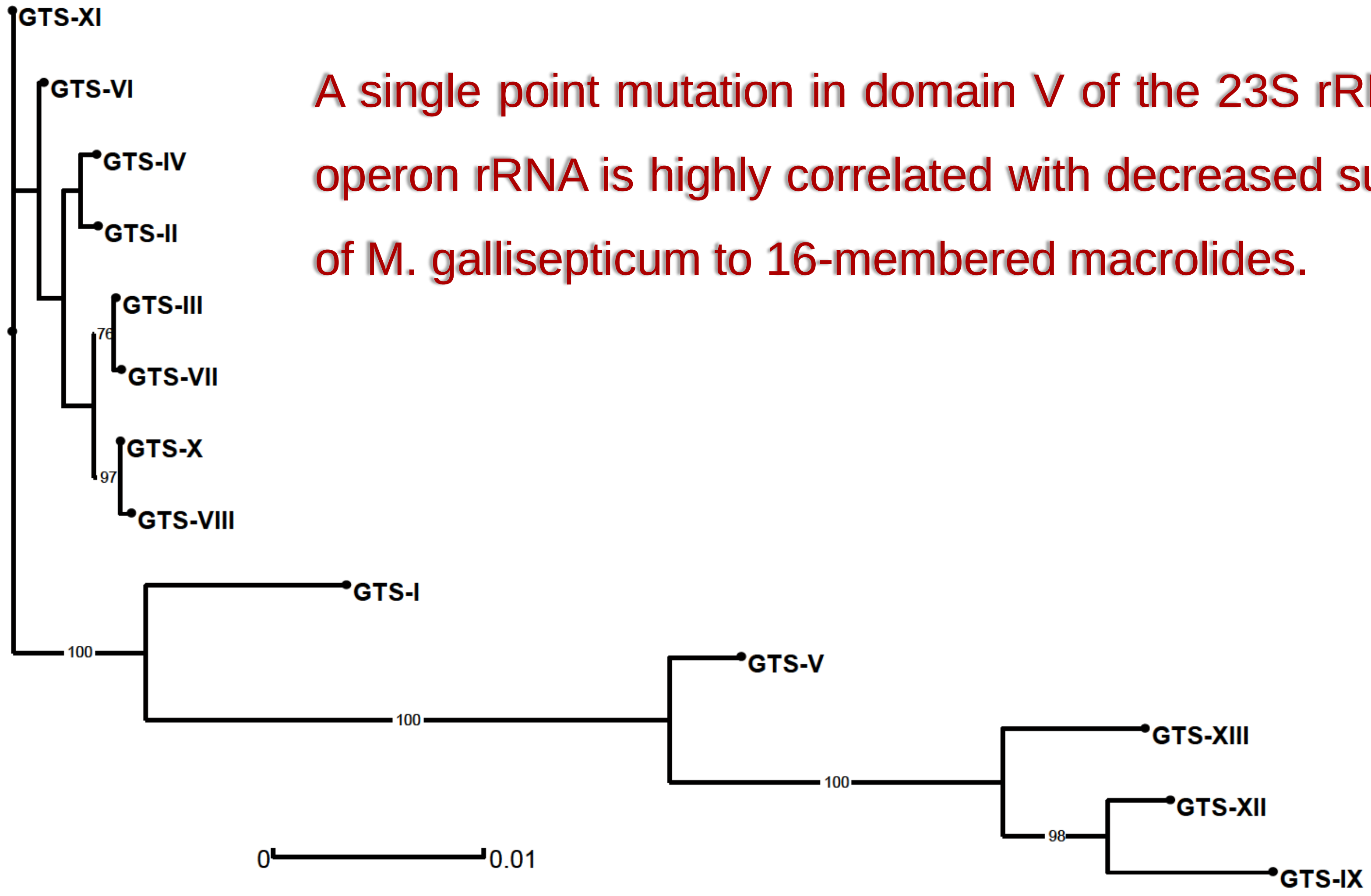
Gharaibeh S, Al-Rashdan, 2011

Characterization of in vivo-acquired resistance to macrolides of Mycoplasma gallisepticum strains isolated from poultry



	Number of isolates with MIC (µg/mL) of														
Antimicrobial agent	**≤0.0032**	**0.0063**	**0.0125**	**0.025**	**0.05**	**0.1**	**0.25**	**0.63**	**1.25**	**2.5**	**5**	**≥10**	**MIC_{50}**	**MIC_{90}**	**% Resistance**
Tylosin	1	11	10	2	1			**3**	**8**	**13**	**1**		0.05	2.5	50
Tilmicosin	16	6		2		1			**3**		**7**	**15**	0.1	≥10	50
Enrofloxacin*				3	10	9	5		**2**	**11**	**8**	**2**	0.25	5	46

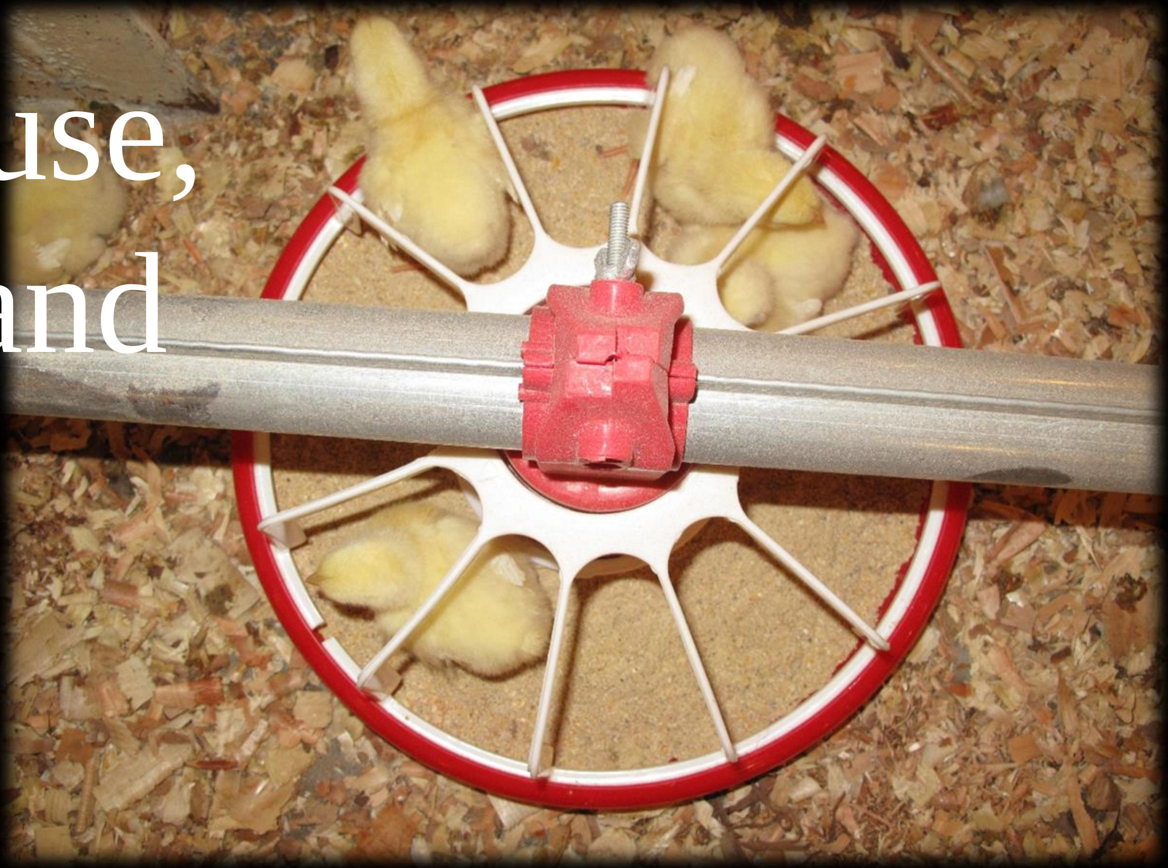
Gerchman et al. 2011



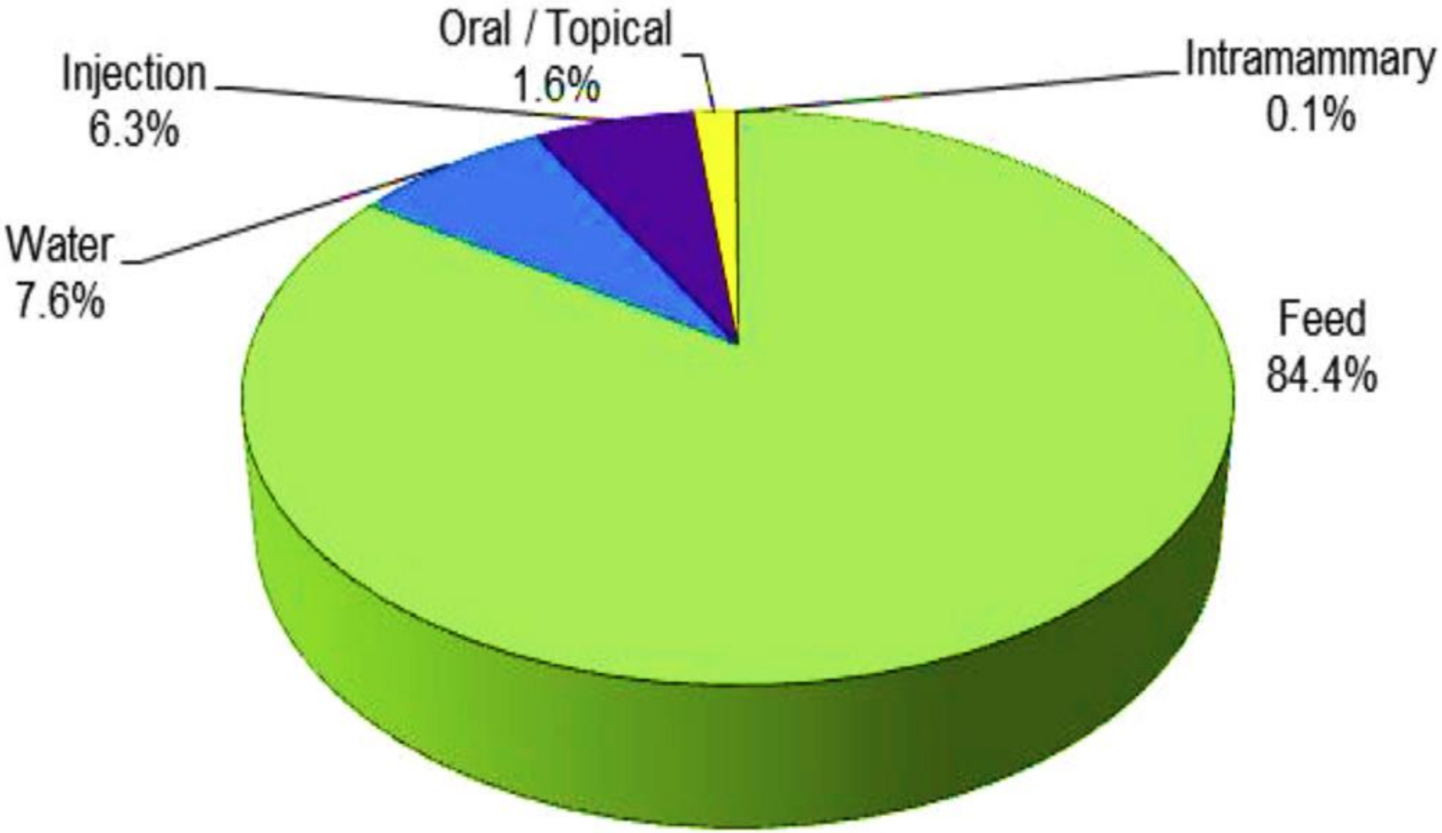
A single point mutation in domain V of the 23S rRNA gene of operon rRNA is highly correlated with decreased susceptibility of M. gallisepticum to 16-membered macrolides.

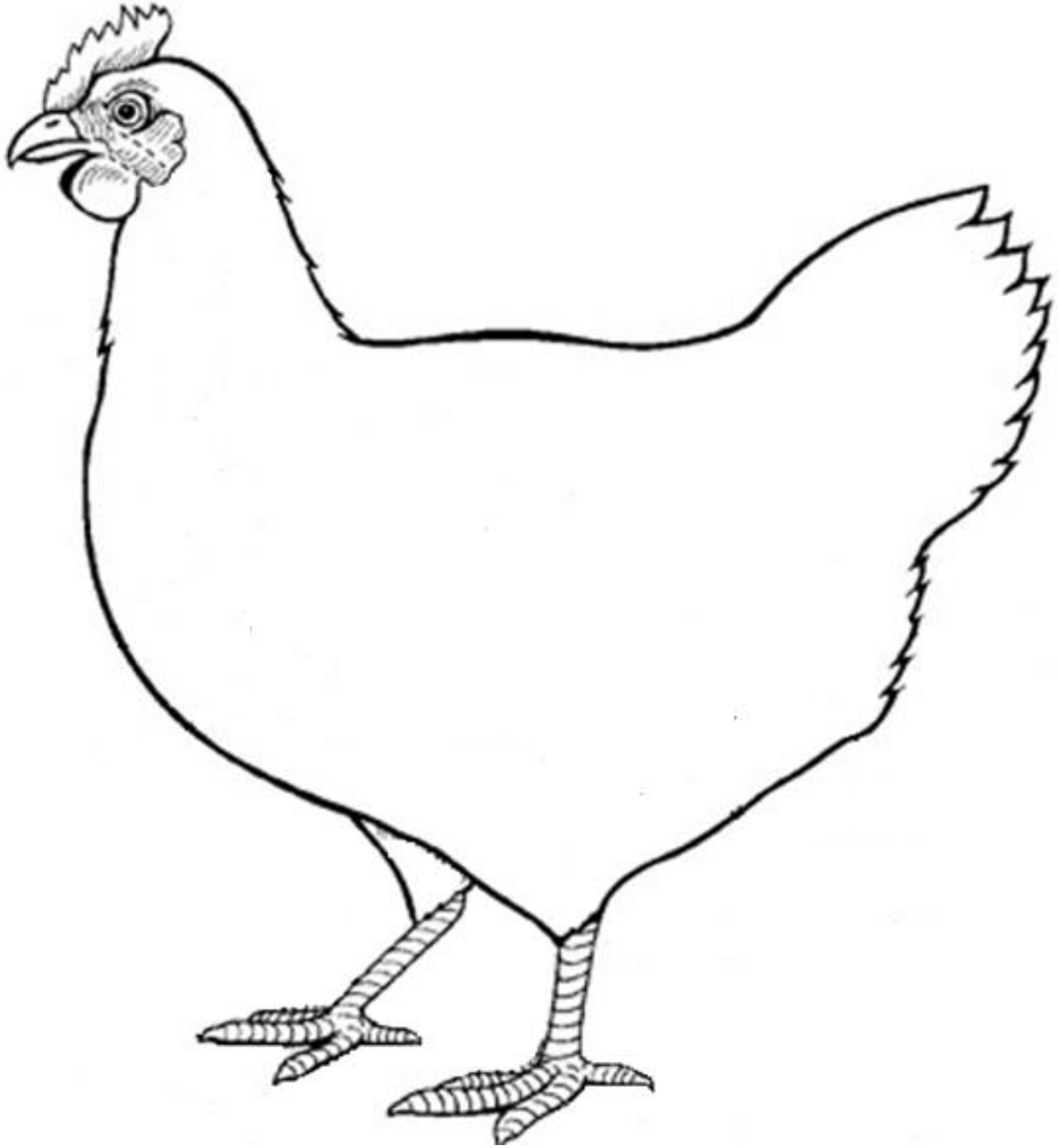
Gerchman et al. 2011

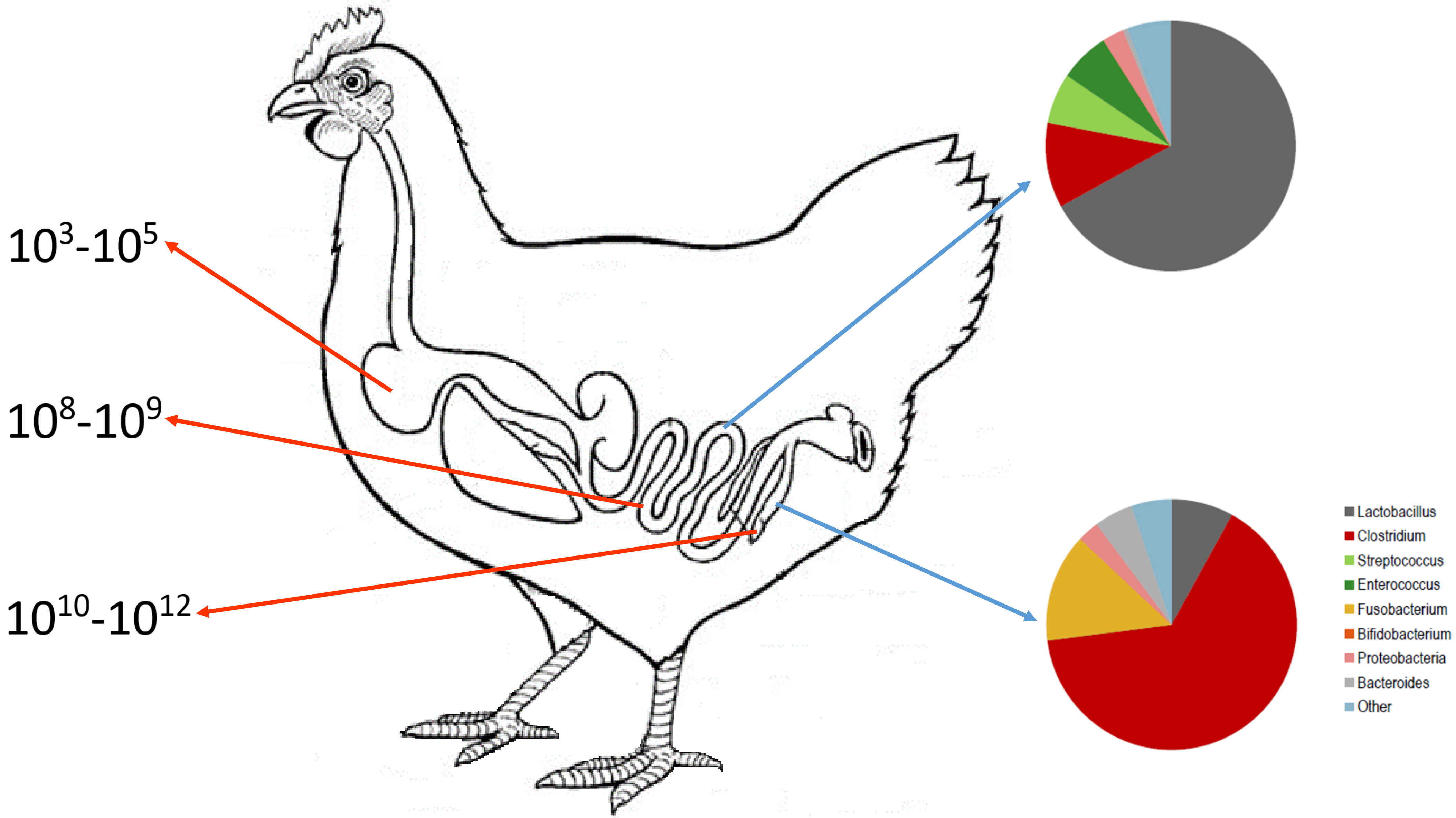
Antibiotic use, resistance and the link to nutrition

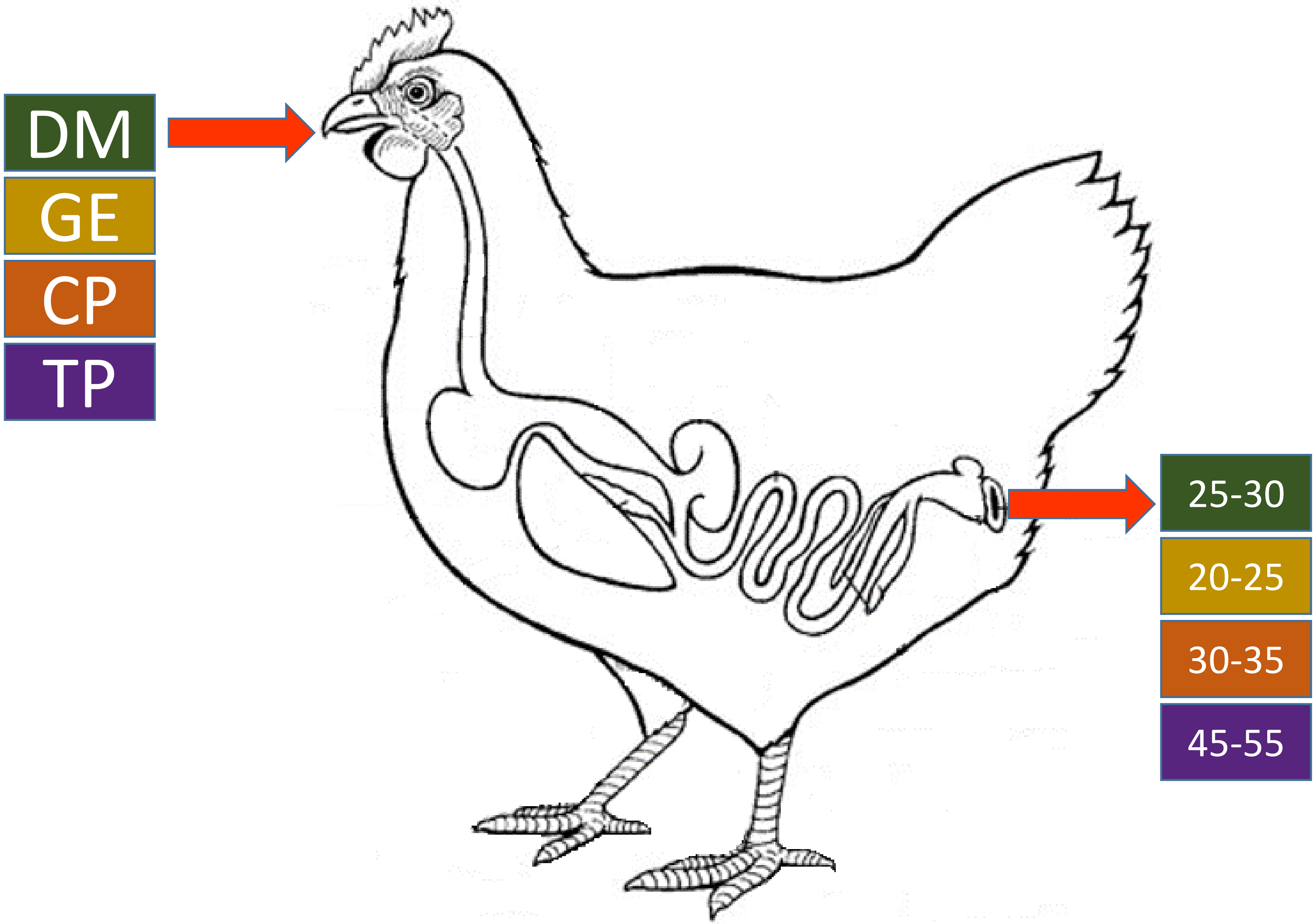


Quantity of antimicrobials (% of total weight in kg) distributed for veterinary use by route of administration in Canada (CSCRA, 2016).

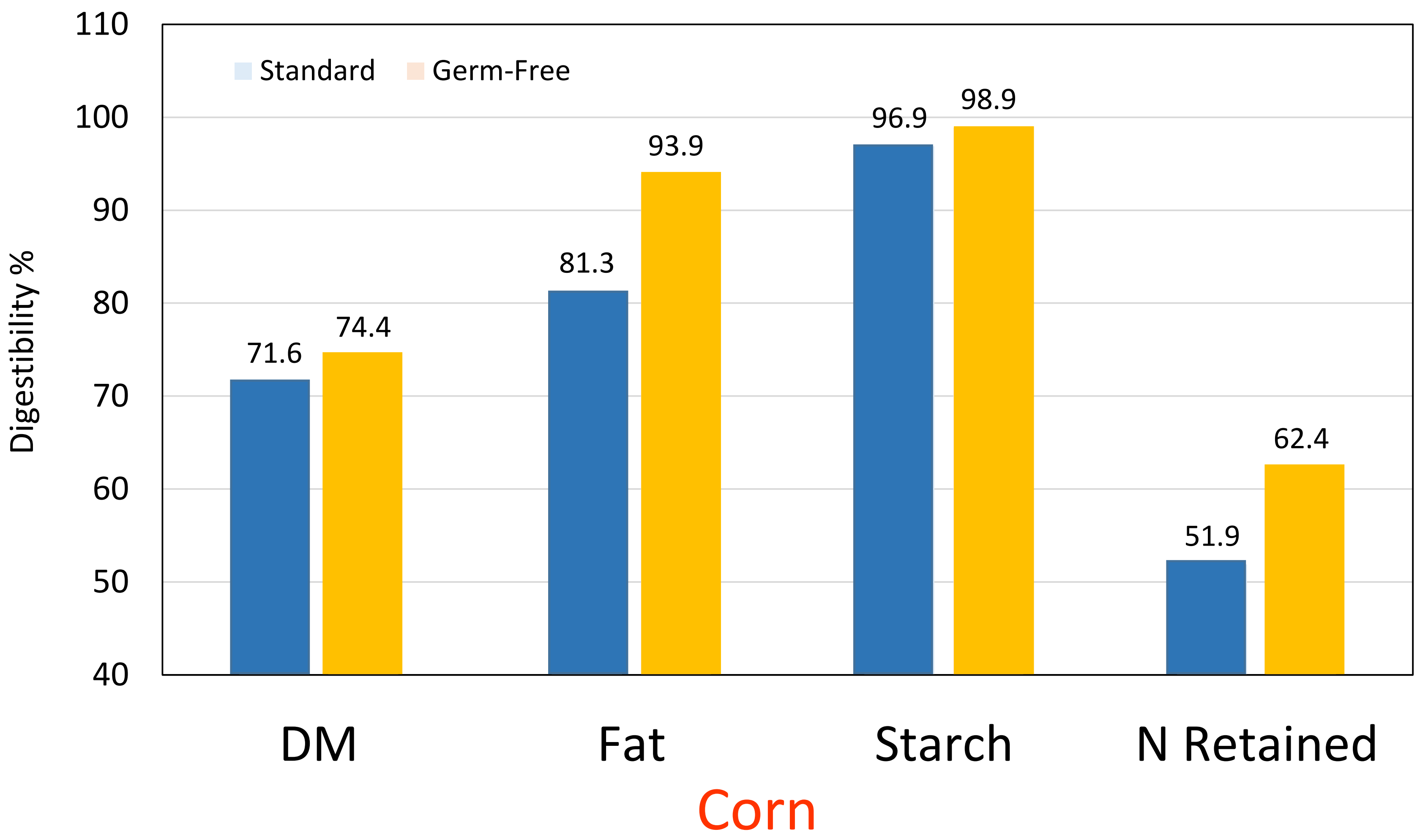




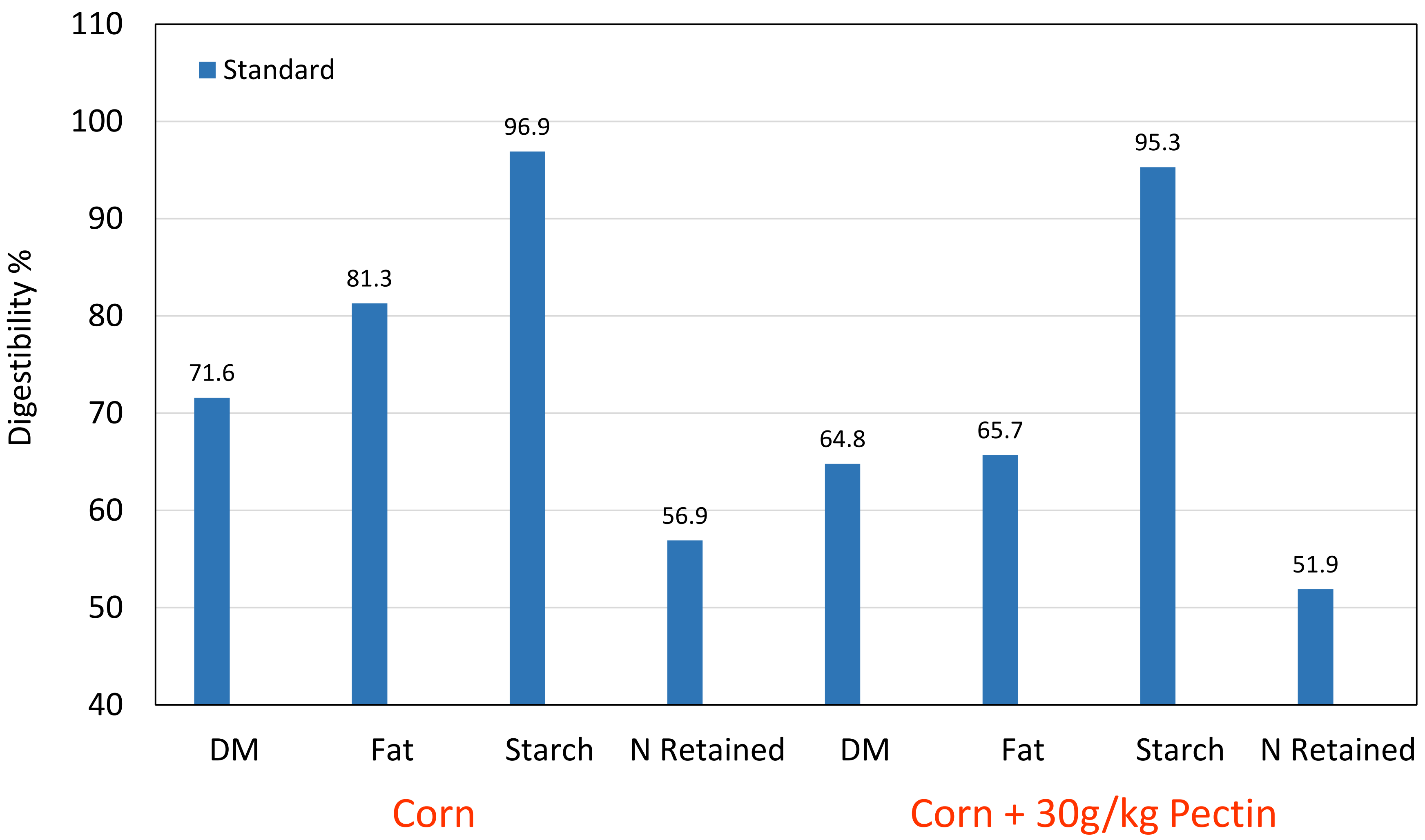

10^3-10^5
10^8-10^9
10^{10}-10^{12}
Lactobacillus
Clostridium
Streptococcus
Enterococcus
Fusobacterium
Bifidobacterium
Proteobacteria
Bacteroides
Other


DM
GE
CP
TP
25-30
20-25
30-35
45-55

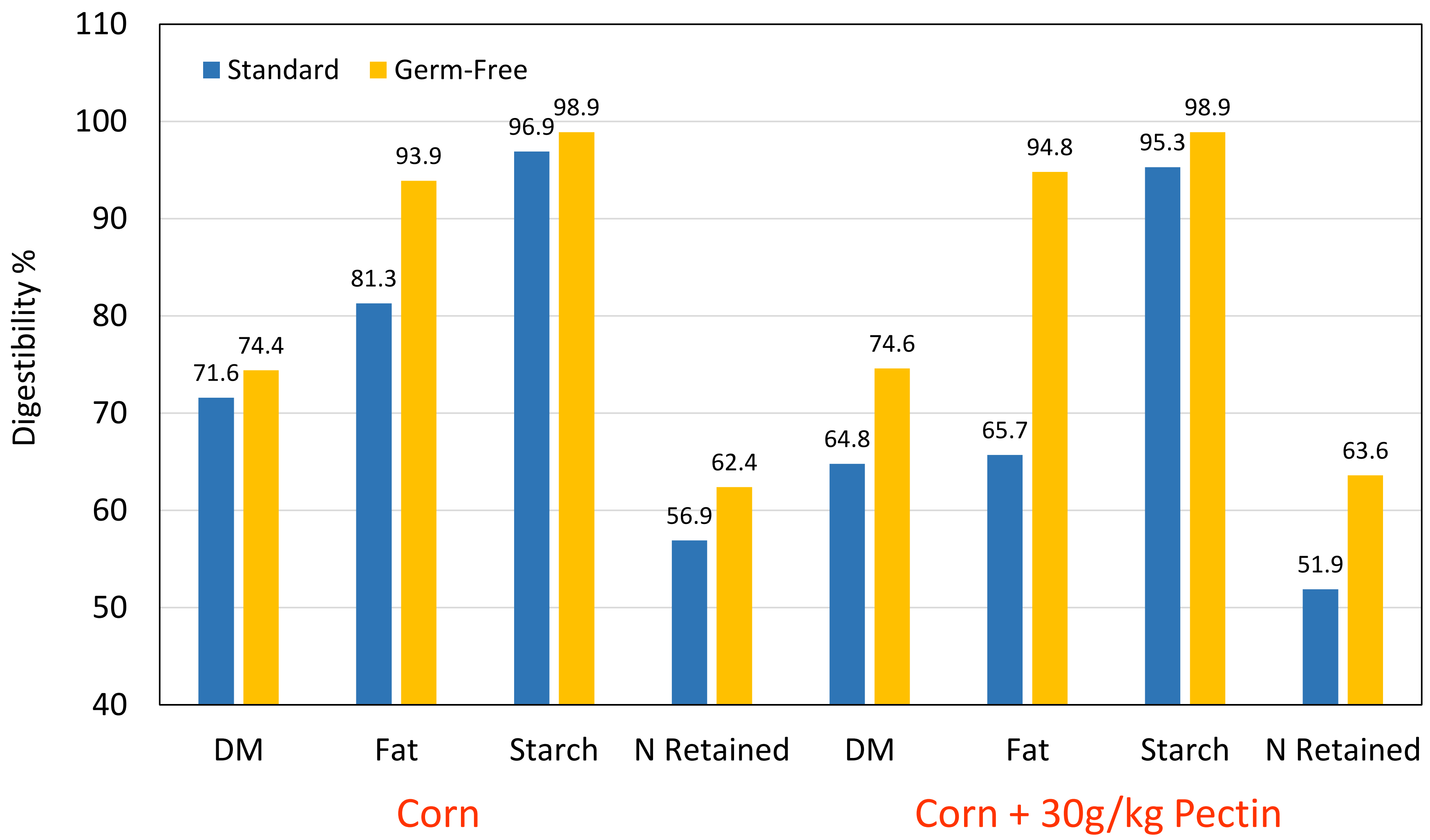
Feed Interacts with Bacteria



Feed Interacts with Bacteria



Feed Interacts with Bacteria



Microbial competition for dietary protein in chicken small intestine

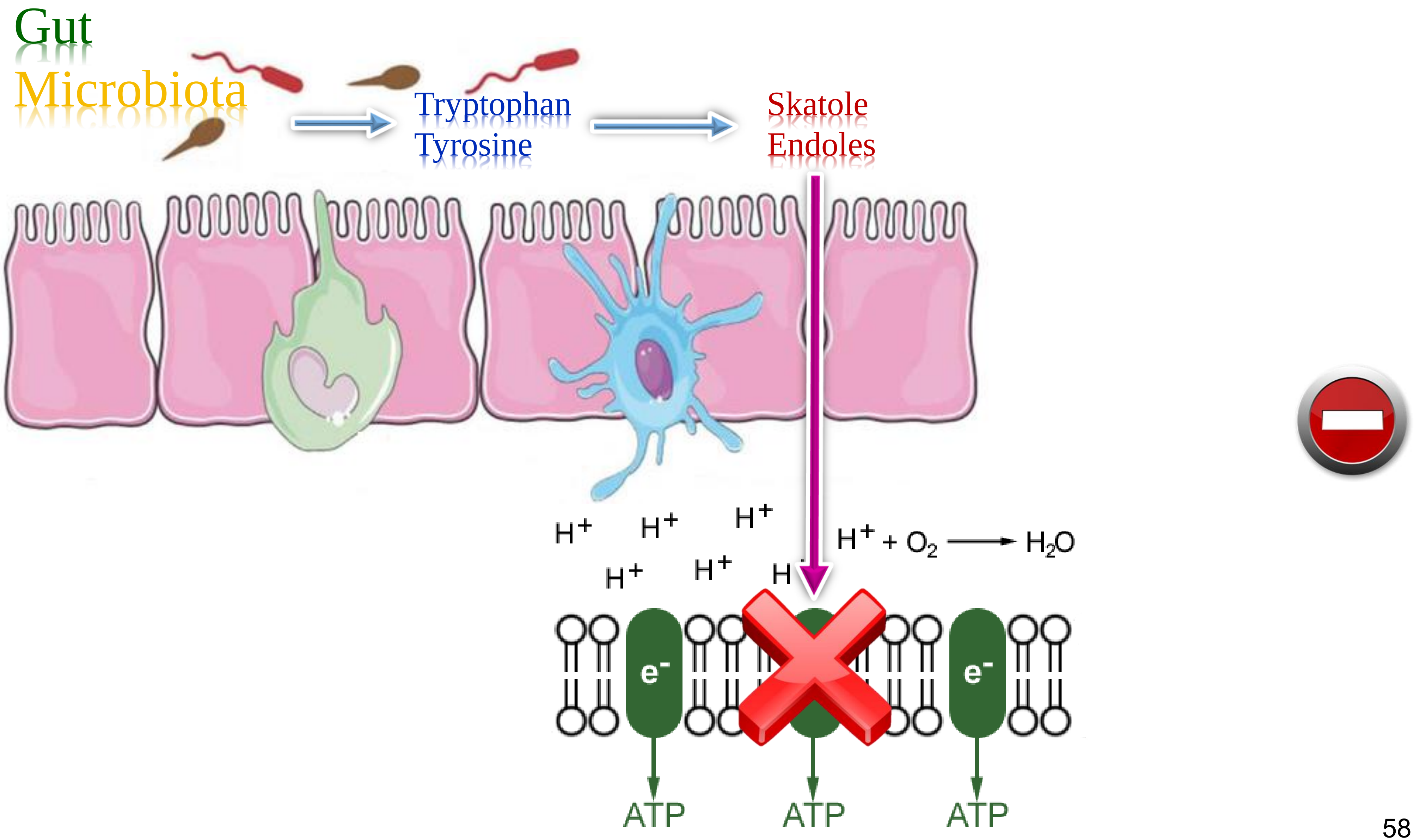
Amino acid requirements of some small intestine bacteria.

	Lactobacillus spp.[a]	*Clostridium perfringens*[b]	*Escherichia coli*
Alanine	±	–	–
Arginine	+	+	–
Aspartic acid	+	–	–
Cysteine	+	–	–
Glutamic acid	+	+	–
Glycine	±	–	–
Histidine	±	+	–
Iso-leucine	+	+	–
Leucine	+	+	–
Lysine	±	–	–
Methionine	+	+	–
Phenylalanine	+	+	–
Proline	±	–	–
Serine	±	–	–
Threonine	+	+	–
Tryptophan	+	+	–
Tyrosine	+	+	–

+growth of the tested strains was dependent on the amino acid
±growth of some of the tested strains was dependent on the amino acid
–growth of the tested strains was not dependent on the amino acid

[a] Morishita et al., 1981.
[b] Sebald and Costilow, 1975.

J. Apajalahti, K. Vienola (2016)


Gut
Microbiota
Tryptophan
Tyrosine
Skatole
Endoles
H+
H+
H+
H+
H+
H
H+ + O2
H2O
e-
e-
ATP
ATP
ATP

Cell

Article

Microbial Reconstitution Reverses Maternal Diet-Induced Social and Synaptic Deficits in Offspring

Shelly A. Buffington,[1,2] Gonzalo Viana Di Prisco,[1,2] Thomas A. Auchtung,[3,4] Nadim J. Ajami,[3,4] Joseph F. Petrosino,[3,4] and Mauro Costa-Mattioli[1,2,*]

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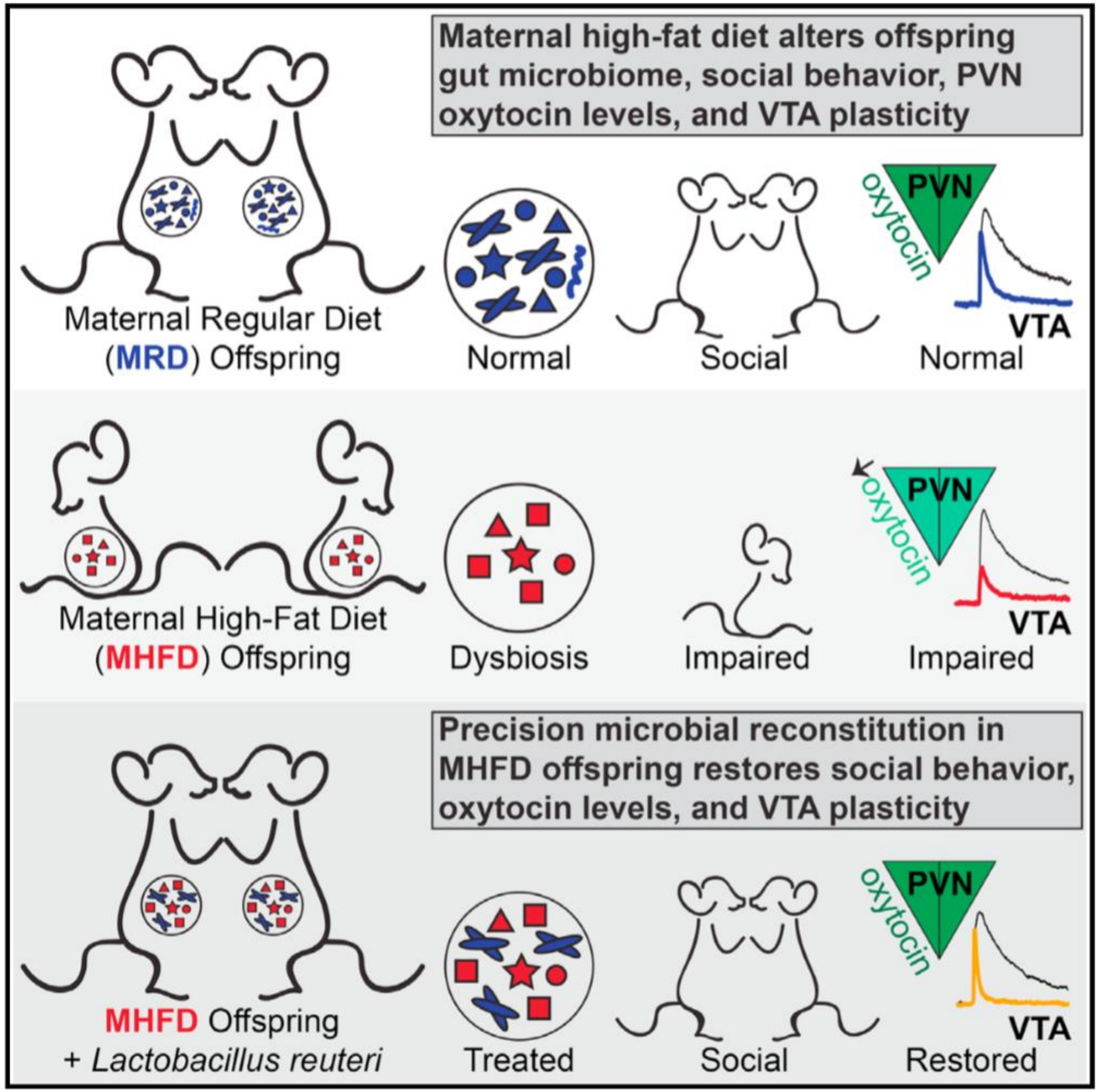
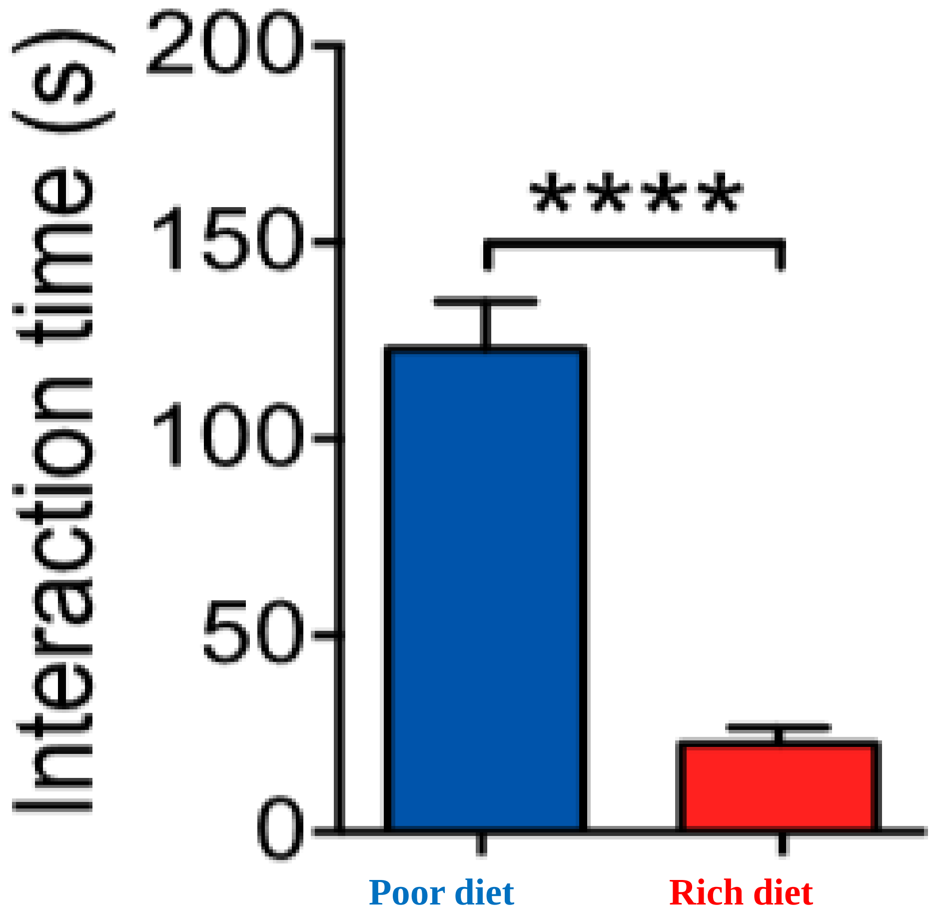
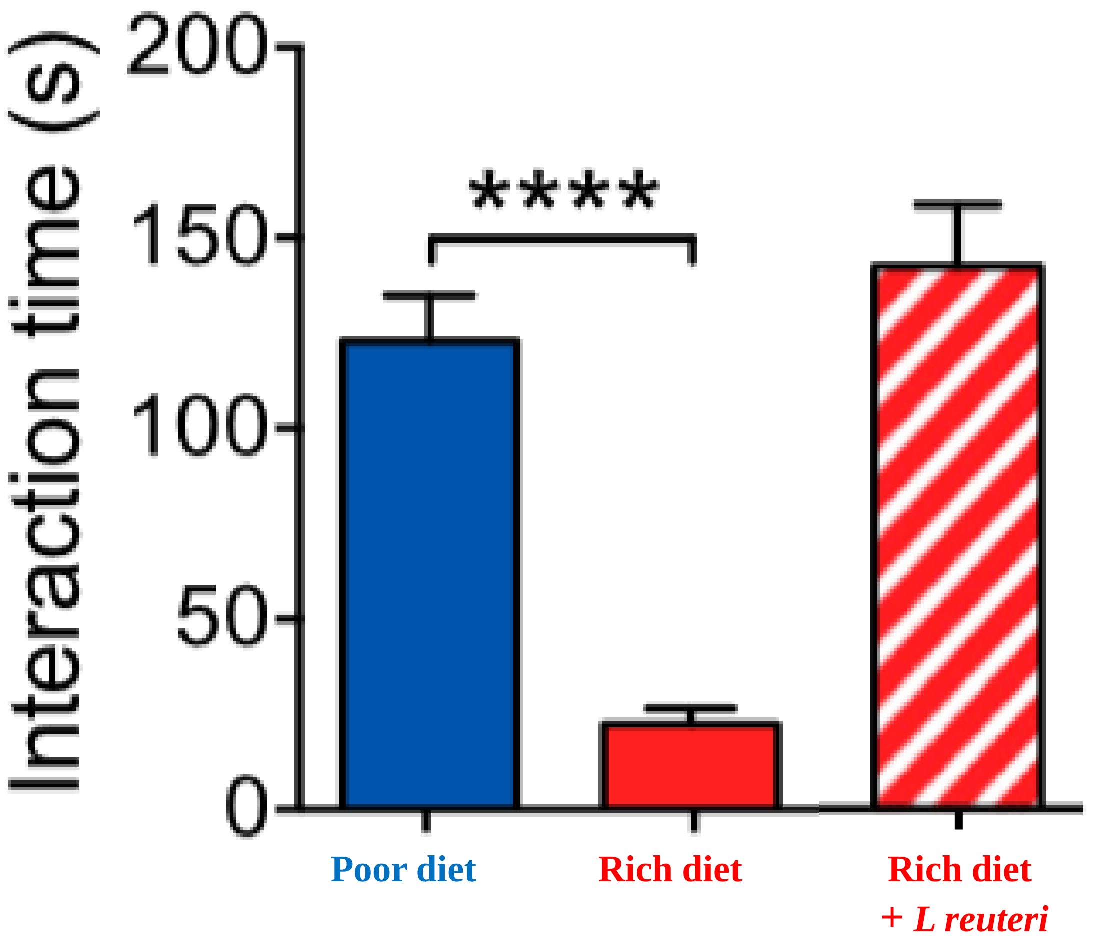
http://dx.doi.org/10.1016/j.cell.2016.06.001

Graphical Abstract




Interaction time (s)
200
150
100
50
0
****
Poor diet
Rich diet


Interaction time (s)
200
150
100
50
0
****
Poor diet
Rich diet
Rich diet
+ L reuteri

Antibiotics

- Reduction in the stability of the microbiota
- Increases Firmicutes/Bacteroidetes
- Fewer functions associated with carbohydrate transport and metabolism
- Smaller arsenal of families involved in the degradation of
  starch,
  cellulose,
  and hemicellulose.

- Lan et al., 2005;
- Danzeisen et al., 2011;
- Allen and Stanton, 2014;
- Mancabelli et al., 2016 ;
- Clavijo et al., 2018.

Some probiotics strains carry antibiotic resistance genes

Ivan & Joyce, 2016