

# **Molecular Understanding of Acquired Antibiotic Resistance in *Mycoplasma gallisepticum* and *Mycoplasma synoviae* and its Implications in the Field**

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# General Information

## What are antibiotics?

Antibiotics are medicines used to treat or prevent infections caused by bacteria.

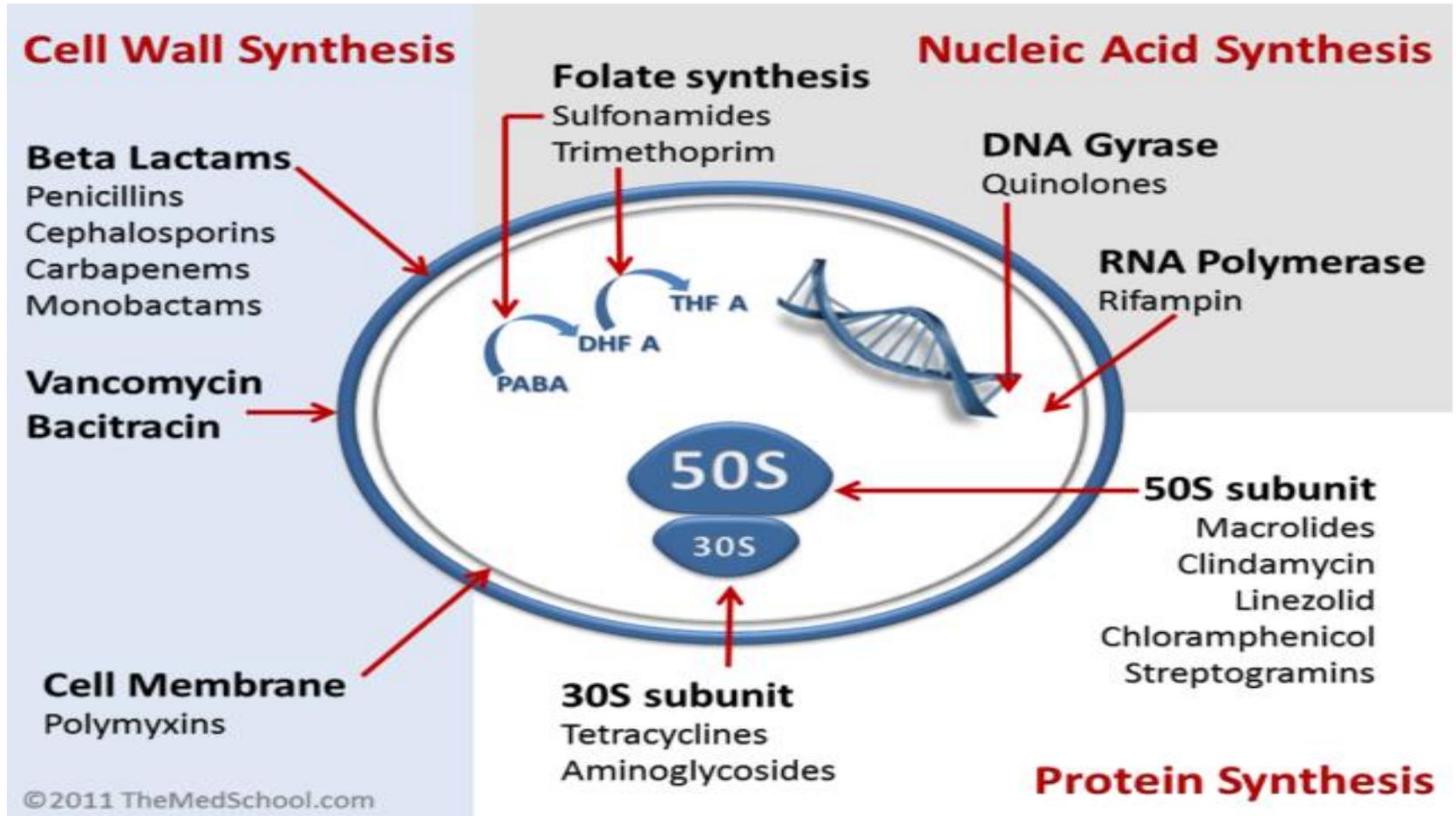
## How do antibiotics work?

Antibiotics work by blocking vital processes in bacteria, killing the bacteria (bactericidal), or stopping them from multiplying (bacteriostatic).

## What is antimicrobial resistance?

Antimicrobial resistance is a lack of susceptibility to an antimicrobial drug.

# Modes of Antibiotic Action



# Antibiotic Resistance

Intrinsic (Natural)

Some microorganisms may be “born” resistant

Acquired



# Intrinsic Resistance to Antimicrobial Agents

## Lack of target

Cell wall

**$\beta$ -lactams**  
**Glycopeptides**

LPS

Dihydropteroate synthetase

Dihydrofolate reductase

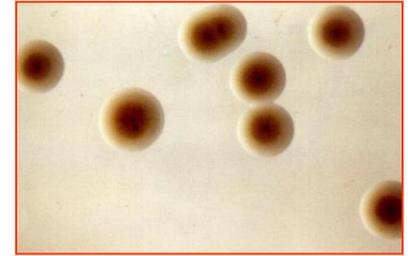


**Polymyxins**  
**Sulfonamides**  
**Trimethoprim**

## Naturally insensitive target

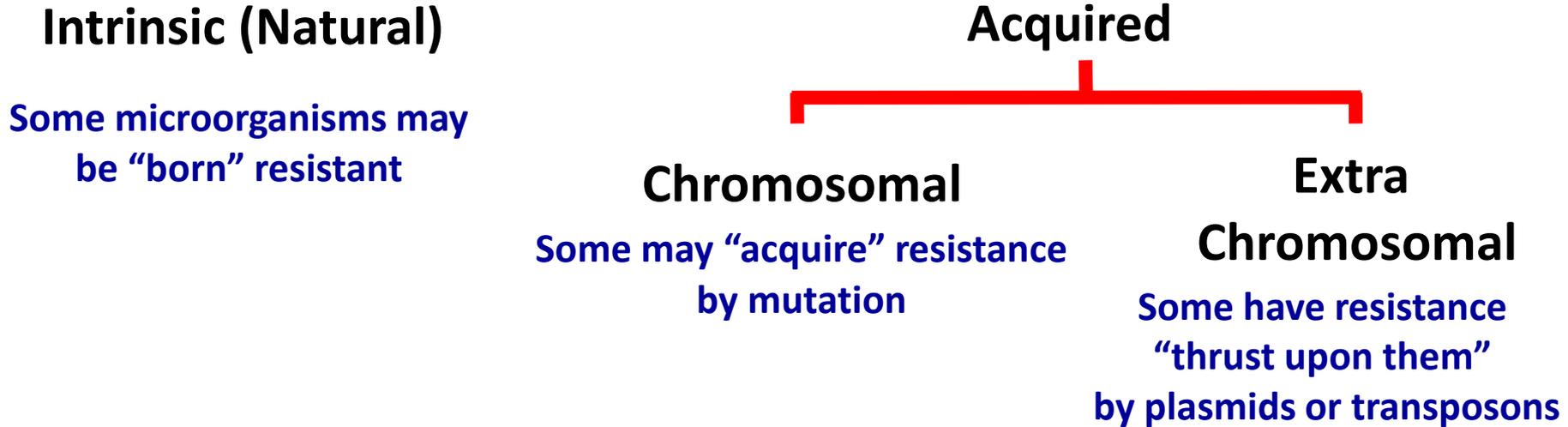
$\beta$  subunit of bacterial RNA polymerase (*rpoB*)

**Rifampin**



**Some species have intrinsic resistance to certain drugs within a class**

# Antibiotic Resistance



# Acquired Antibiotic Resistance in *M. gallisepticum* and *M. synoviae*

Antimicrobials	MIC Range ( $\mu\text{g/ml}$ )	
	MG	MS
<b>Tetracyclines</b>		
Tetracycline	0.08-0.16	0.08-2
Oxytetracycline	$\leq 0.031$ ->16	0.025->100
Chlortetracycline	0.2-32	0.39->12.5
Doxycycline	$\leq 0.031$ ->80	0.015-0.125
<b>MLSK group</b>		
Erythromycin	$\leq 0.03$ ->80	4- $\geq 128$
Tylosin	0.0025->256	$\leq 0.0025$ -50
Tilmicosin	$\leq 0.0125$ ->25	0.006- $\geq 8$
Kitasamycin	0.03-11	0.4-5.6
Josamycin	$\leq 0.03$ ->50	0.125-1.5
Spiramycin	0.02->20	0.04-1.25
Lincomycin	0.1->256	0.1-8
<b>Pleuromutilins</b>		
Tiamulin	0.0005->256	$\leq 0.03$ -1
Valnemulin	$\leq 0.008$ ->64	$\leq 0.004$ -0.16
<b>Fluoroquinolones</b>		
Enrofloxacin	0.01-10	0.024-16
Danofloxacin	0.01-0.5	0.064-1
Flumequine	2.5->10	5-64
Difloxacin	0.025-5	<0.015-10
<b>Aminoglycosides</b>		
Gentamycin	1- $\geq 64$	1
Apramycin	>16	2-4
Spiramycin	0.25-10	ND
<b>Aminocyclitol</b>		
Spectinomycin	$\leq 0.031$ -12.5	0.5-2
<b>Phenicols</b>		
Thiamphenicol	0.125-4	ND
Florfenicol	0.125-4	ND

There are no standardized testing methods, quality control guidelines and interpretative MIC breakpoints for mycoplasmas of food animals.



**Hard to compare results from different laboratories**

Only a limited number of studies relating to the *in vitro* susceptibility of MG and MS field strains have been published.



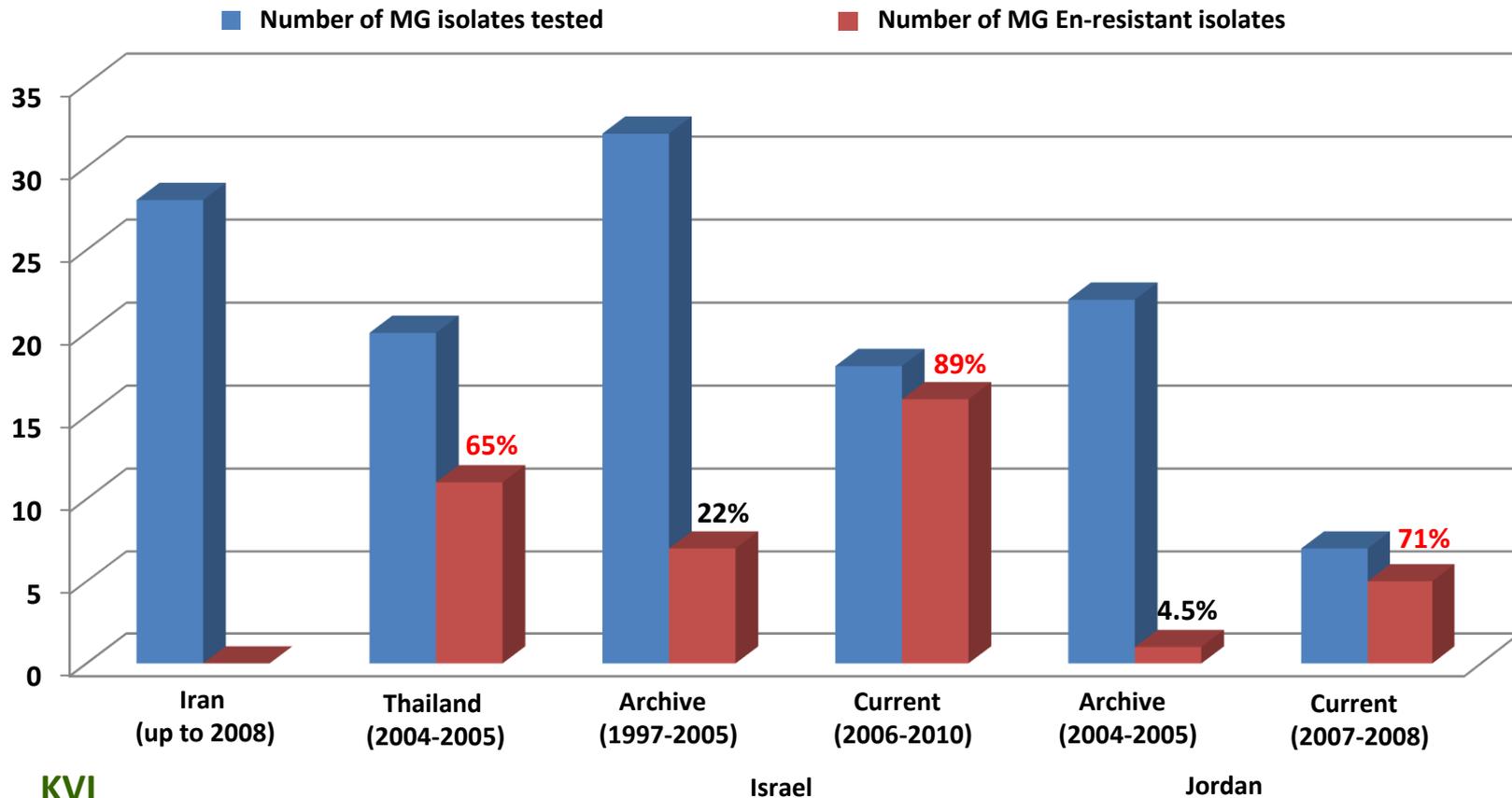
**Hard to get a global picture and trends**

# Variations in the Proportion of *M. gallisepticum* and *M. synoviae* Isolates with Decreased Susceptibility

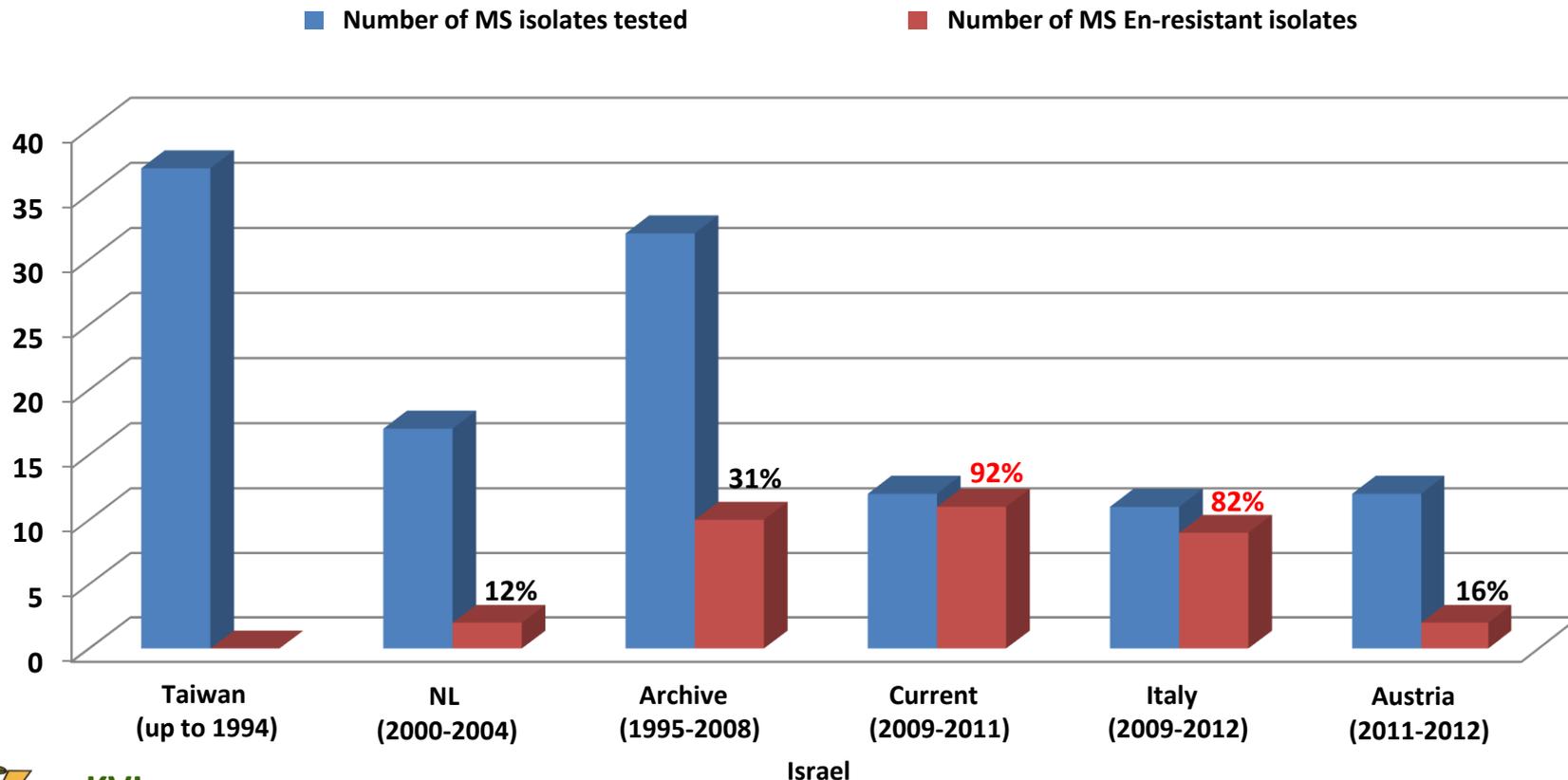
## Related to:

- Geographical origin
- Year of isolation
- Type of livestock production system
- Clinical presentation of the strains tested
- Differences among the countries in regulatory practices for use of antimicrobials

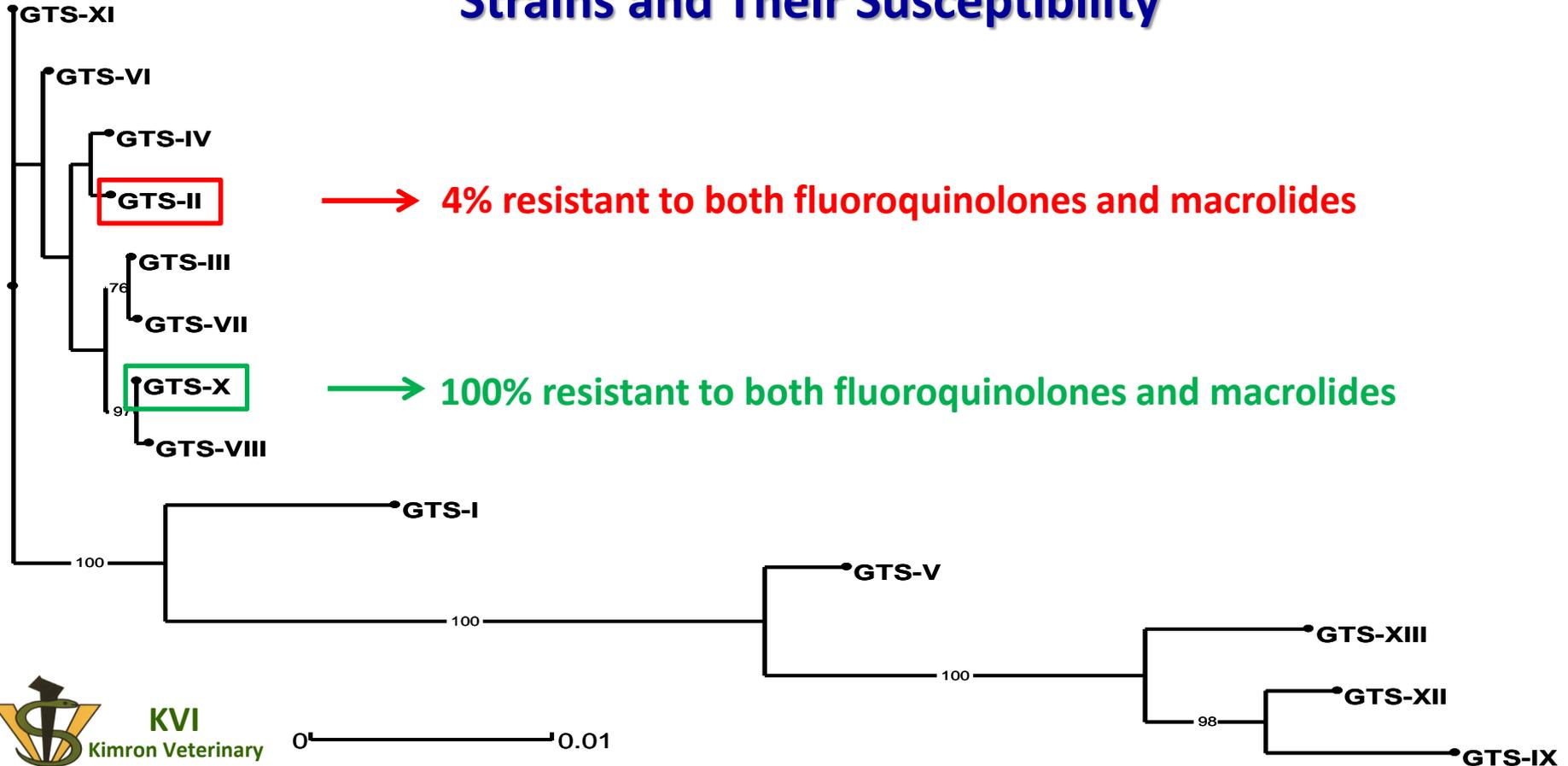
# *M. gallisepticum* Isolates With Decreased Susceptibility to Fluoroquinolones



# *M. synoviae* Isolates With Decreased Susceptibility to Fluoroquinolones



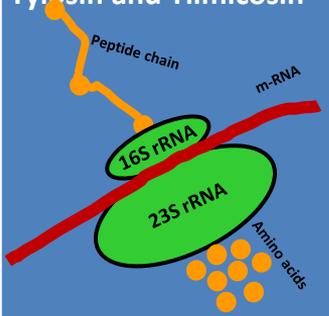
# Genetic Variability of Israeli *M. gallisepticum* Strains and Their Susceptibility



# Mechanisms of Acquired Resistance Reported in MG and MS

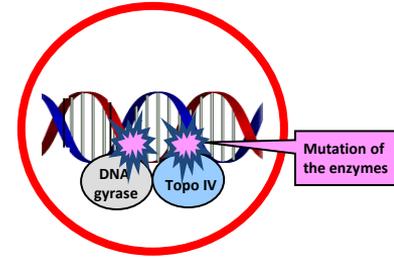
**MLSK resistance:** point mutations within the macrolide-binding sites (II and V) located in the 23S rRNA genes

Antimicrobials	Mechanism of acquired resistance	MIC of resistant isolates ( $\mu\text{g/ml}$ )	Mechanism of acquired resistance	MIC of resistant isolates ( $\mu\text{g/ml}$ )
	<b>MS</b>		<b>MG</b>	
Erythromycin	Presence G instead of A at position 2057 in domain V of the 23S rRNAs ( <u>intrinsic resistance</u> )	$\geq 32$	Mutations at positions 2057, 2058, 2059 in domain V of the 23S rRNA ( <i>in vitro</i> )	$\geq 256$
Tylosin and Tilmicosin	<p>Mutations at position 748 in domain II of 23S rRNA caused a slight increase in MICs</p> <p>Mutations at positions 2058 or 2059 in domain V of 23S rRNA correlated with a more significant decrease in susceptibility</p>	<p>Ty: up to 0.5 Tm: up to 2</p> <p>Ty: 1-2 Tm <math>\geq 8</math></p>	Mutations at positions 2058, 2059 in domain V of 23S rRNA ( <i>rrnA</i> , MGA_01)	<p>Ty: 0.63-<math>\geq 10</math> Tm: 1.25-<math>\geq 10</math></p>

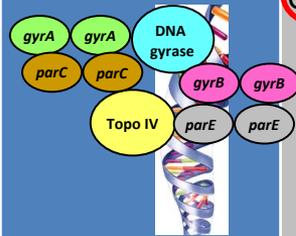


# Mechanisms of Acquired Resistance Reported in MG and MS

**Fluoroquinolone resistance:** point mutations within the (QRDRs) of DNA gyrase subunits GyrA and GyrB and/or topoisomerase IV subunits ParC and ParE



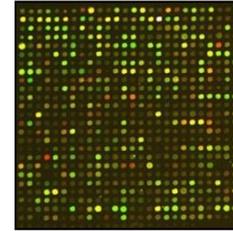
Antimicrobial class	Mechanism of acquired resistance	MIC of resistant isolates (µg/ml)	Mechanism of acquired resistance	MIC ranges for resistant isolates (µg/ml)
	<b>MG</b>		<b>MS</b>	
<b>Fluoroquinolones</b>				
<b>Enrofloxacin</b>	Mutations in the QRDRs of <b>GyrA</b> and ParC	1->10	Mutations in the QRDR of <b>ParC</b> The relevance of the mutations in the GyrA, GyrB genes should be clarified in the future	1-16



**Tetracycline and aminoglycoside:** the genetic basis for decreased susceptibilities have not been elucidated

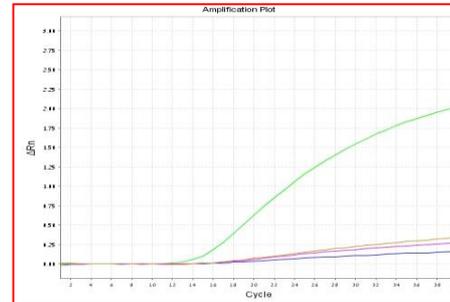
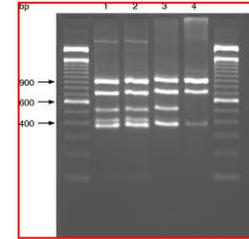
# Genetic Methods for the Detection of Resistance

**Nucleic acid hybridization:** (micro- and macroarrays)



**Nucleic acid amplification:**

simple or multiplex PCR (with/ without RFLP)  
real-time PCR  
real-time RT-PCR (gene expression)



# Phenotypic and Genotypic Methods for the Detection of Susceptible or Resistant Strains: **Benefits and Constraints**

Methods	Rapidity	Unavailability to obtained isolates by culture	Cost	Emerging new resistance mechanisms
<b>Phenotypic</b>	Cannot be performed directly on clinical sample; time consuming	Non useful	Relatively cheap	Yes
<b>Genotypic</b>	<b>Can be performed directly on clinical sample; rapid</b>	Useful	Can be expensive when screening for multiple resistance determinants	<b>No; screen exclusively for known mechanisms</b>

# Genetic Methods Screen Exclusively for Known Mutations and Mechanisms

JOURNAL OF CLINICAL MICROBIOLOGY, Aug. 2010, p. 2909–2915  
0095-1137/10/\$12.00 doi:10.1128/JCM.00699-10  
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Vol. 48, No. 8

## Development and Evaluation of a Novel Single-Nucleotide-Polymorphism Real-Time PCR Assay for Rapid Detection of Fluoroquinolone-Resistant *Mycoplasma bovis*<sup>▽†</sup>

M. Ben Shabat, I. Mikula, I. Gerchman, and I. Lysnyansky\*

*Mycoplasma Unit, Division of Avian and Fish Diseases, Kimron Veterinary Institute, Bet Dagan, Israel*

- Detection of nt substitution of G to A in QRDR-*parC* resulting in the aa substitution of asparagine for aspartic acid at position 84.
- Presence of additional mutations at other positions of QRDR-*parC* resulting in the aa substitution of Ser81Pro or Ser80Ile (Sato et al., 2013).

**Presence of additional aa options and different “hot spots for fluoroquinolones” hampers the use of the method developed and makes it more difficult to design molecular tests**

# Agreement Between Molecular and Conventional Testing

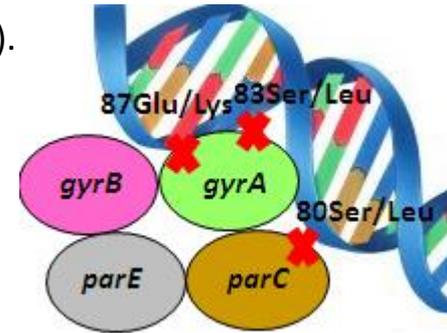
**MG-En resistant strain: concurrent mutations at positions GyrA-83 or GyrA-87 and ParC-80**

**Correlation:** between MIC values and genotypic results was found in **90.3%** (84/93 strains).

**Discrepancy:** between MIC values and genotypic results was found in 9.6% (9/93 strains):

Five strains have → **one of the two mutations** → **resistant** to En

Four strains have → **one of the two mutations** → **susceptible** to En



**Molecular assay targeting both the *gyrA* and *parC* genes can be used as a preliminary rapid screening method for testing En-susceptibility of MG field strains**

## Summary:

- It is necessary to agree on a standardized method and controls for animal mycoplasma antimicrobial susceptibility testing and to develop MIC breakpoints.
- More studies testing *in vitro* susceptibility of current MG and MS field isolates should be performed to get a global picture.
- Any proposed new test must be validated with as many strains as possible originating from different geographic regions.

**Although genetic techniques offer marked advantages, especially in the case of fastidious microorganisms such as mycoplasmas, they are unlikely, at least at this stage, to fully replace the traditional phenotypic tests**

# Acknowledgments



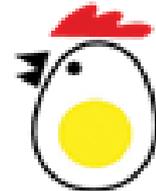
**Sharon Levisohn**



**Irena Gerchman**



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המועצה  
לענף הלול

**Israeli Egg and Poultry Board**