



ISTITUTO G. CAPORALE  
TERAMO

# **Antibiotico resistenza di *Campylobacter* e sierotipi di *C. jejuni* circolanti in Italia**

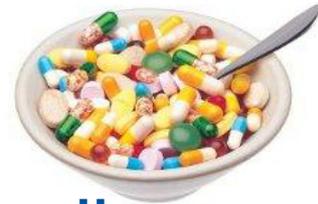
Laboratorio Nazionale di Riferimento per *Campylobacter*  
Lorena Sacchini

Teramo, 12 dicembre 2012

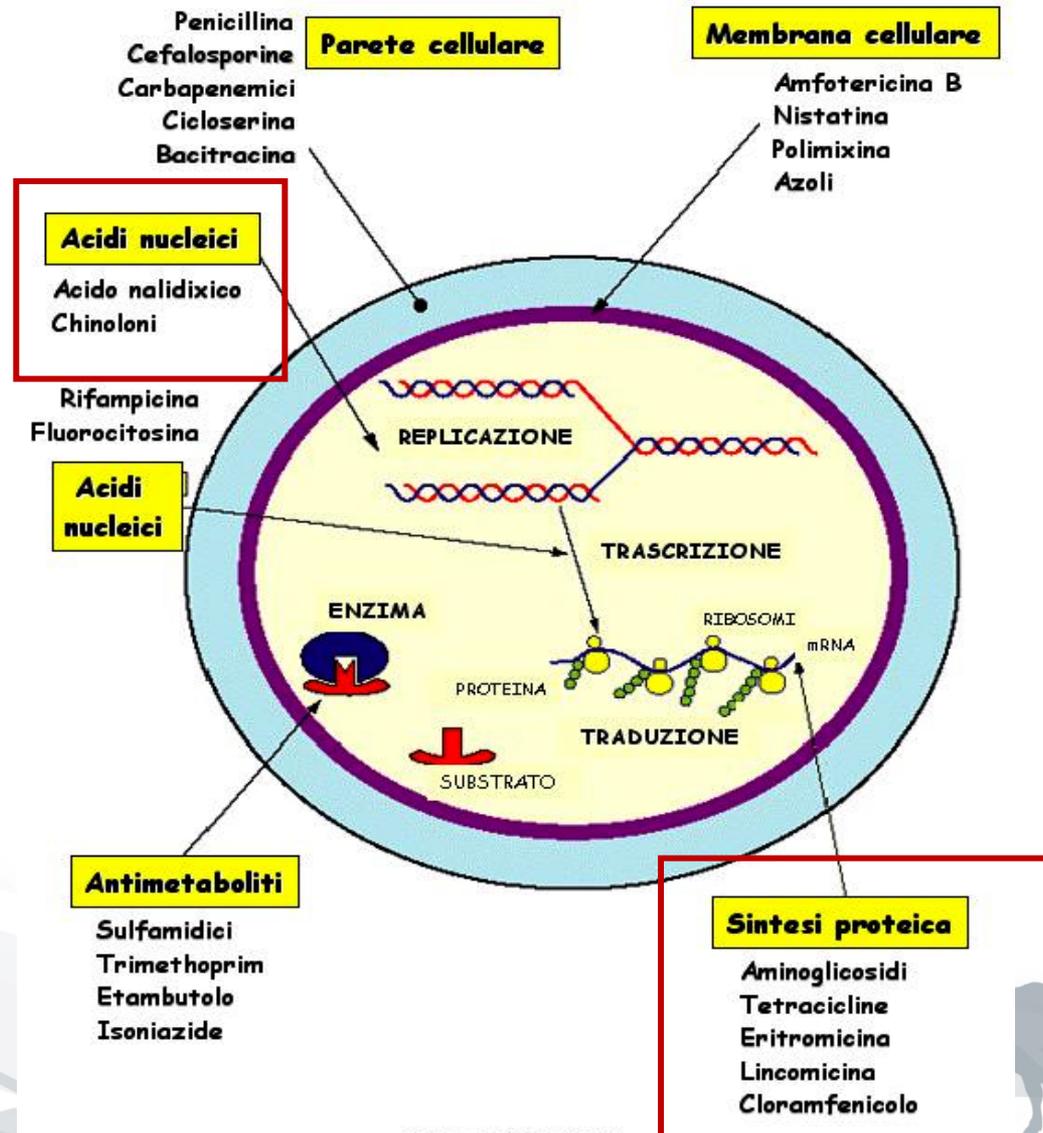


# Normative

- Regolamento CE 1831/2003 vieta dal 2006 l'utilizzo degli antibiotici come additivi nell'allevamento animale.
- Direttiva 2003/99/EC prevede il monitoraggio dell'antibiotico resistenza di *Campylobacter* isolati da pollo, suini, bovini e cibi da essi derivati.
- Decisione 2007/516/CE per attuazione di piani di monitoraggio sulla resistenza agli antimicrobici del *Campylobacter* spp. in polli da ingrasso.



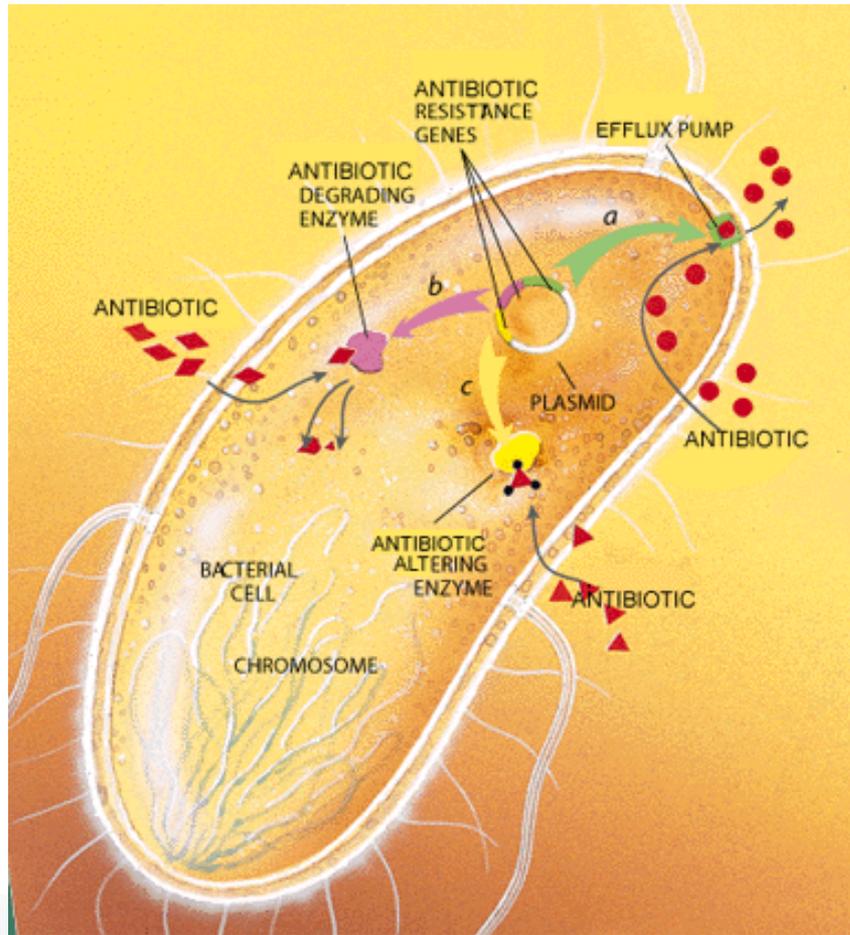
# Meccanismi di azione degli antibiotici





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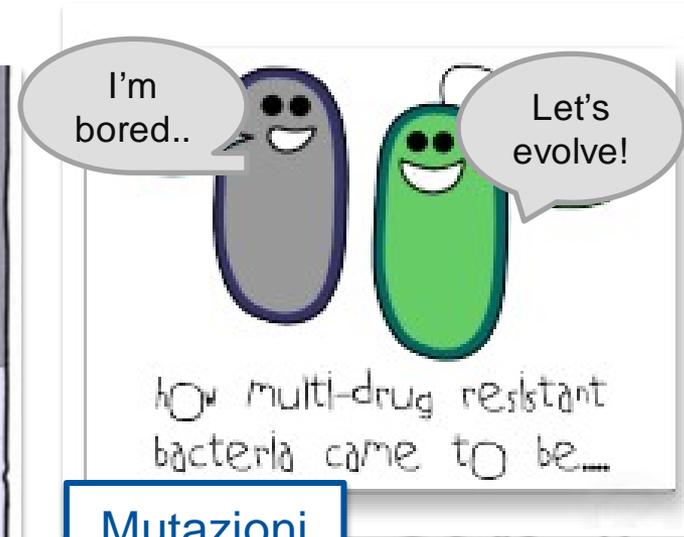
# Meccanismi di resistenza



# Meccanismi di acquisizione della resistenza



Trasferimento materiale genetico

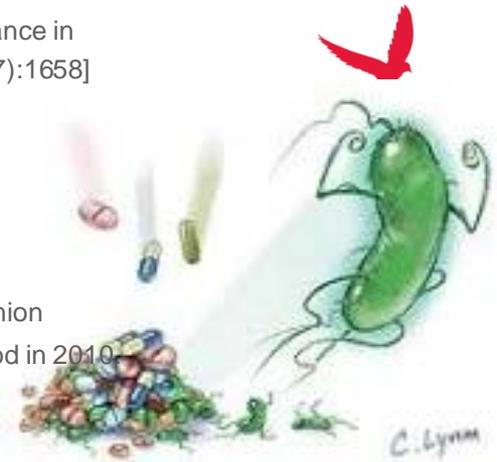


Mutazioni



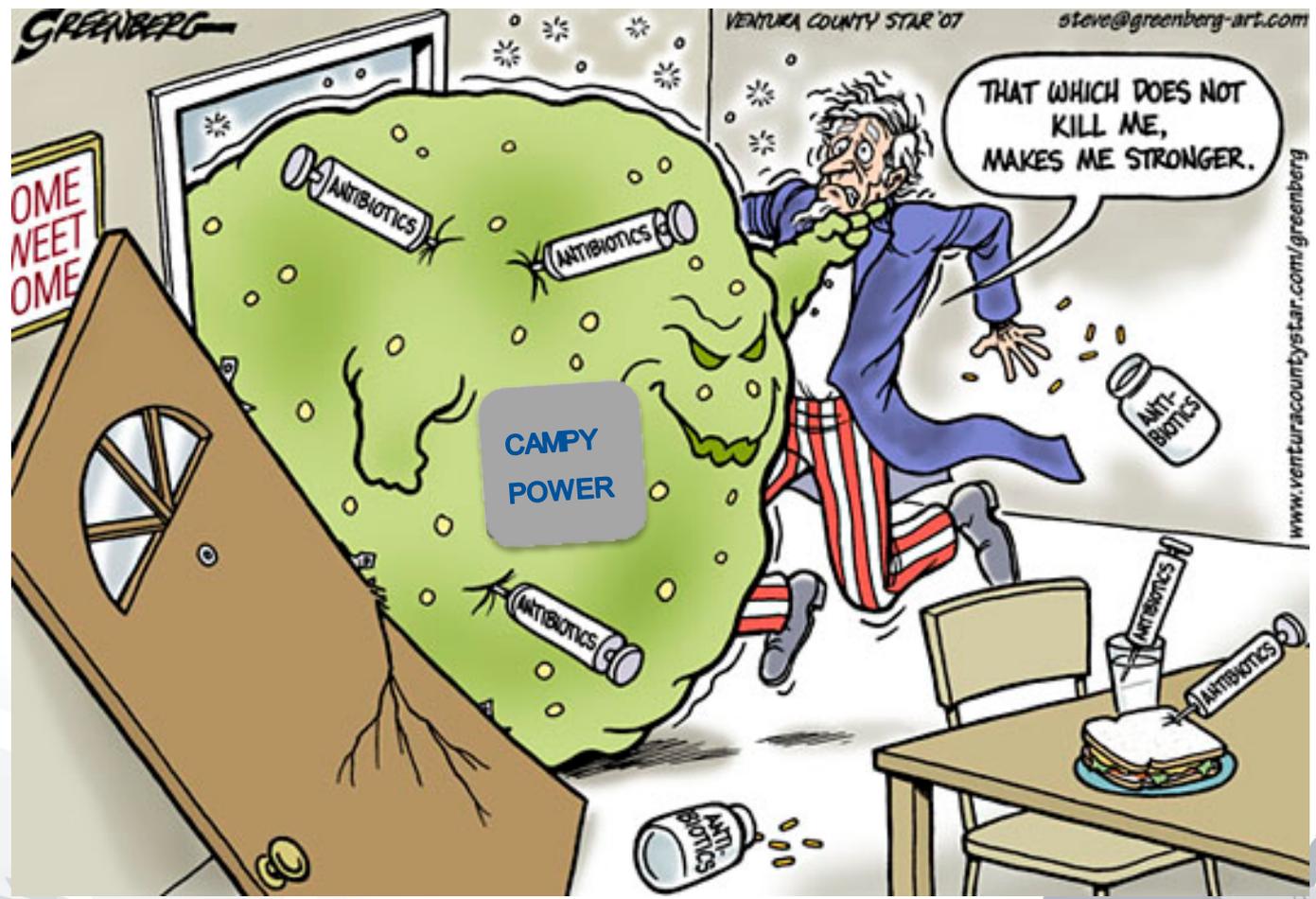
# ...il *Campylobacter* oggi!

- La Campylobacteriosi è la zoonosi più diffusa sul territorio europeo. [EFSA, ECDC: The European Union Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents and Food-borne Outbreaks in the European Union in 2010, EFSA Journal 2012; 10(3):2597]
- Il 20%-30% dei casi è attribuibile alla manipolazione e consumo di carne di pollo, mentre il 50%-80% può essere attribuito al pollo inteso come serbatoio, nel suo complesso (trasmissione ambientale o per contatto diretto). [EFSA: Scientific Opinion on Quantification of the risk posed by broiler meat to human campylobacteriosis in the EU. EFSA Journal 2010; 8(1):1437]
- La resistenza ai fluorochinoloni è un problema di salute pubblica emergente. [EFSA: The Community Summary Report on antimicrobial resistance in zoonotic and indicator bacteria from animals and food in the European Union in 2008. EFSA Journal 2010; 8(7):1658]
- I fluorochinoloni e l'eritromicina rappresentano gli antibiotici d'elezione per il trattamento delle campylobacteriosi in campo umano. [EFSA-ECDC: The European Union Summary Report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2010. EFSA Journal 2012; 10(3):2598]

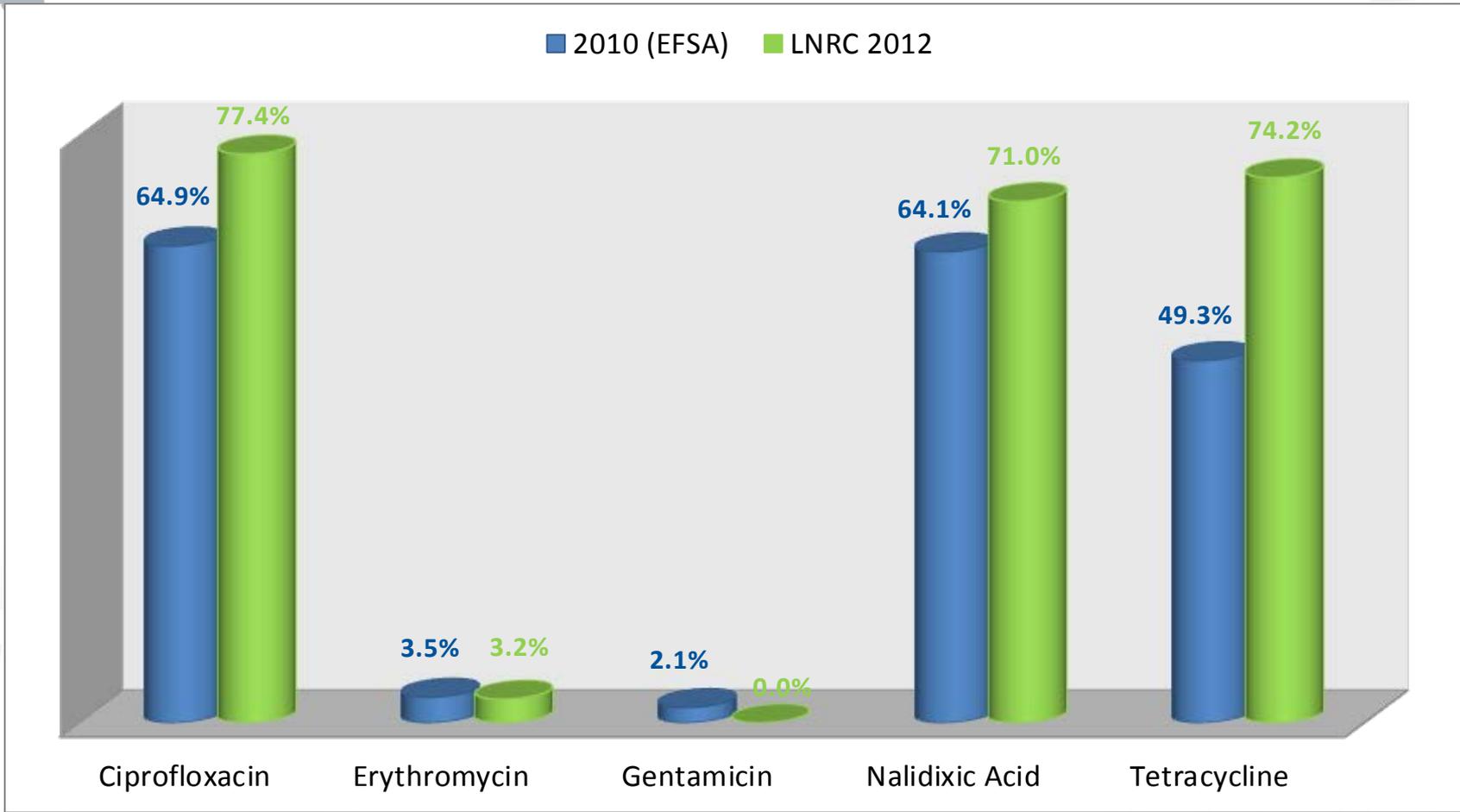




# ...il *Campylobacter* oggi!

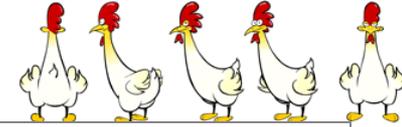


# Resistenza in ceppi di *C. jejuni* di origine umana

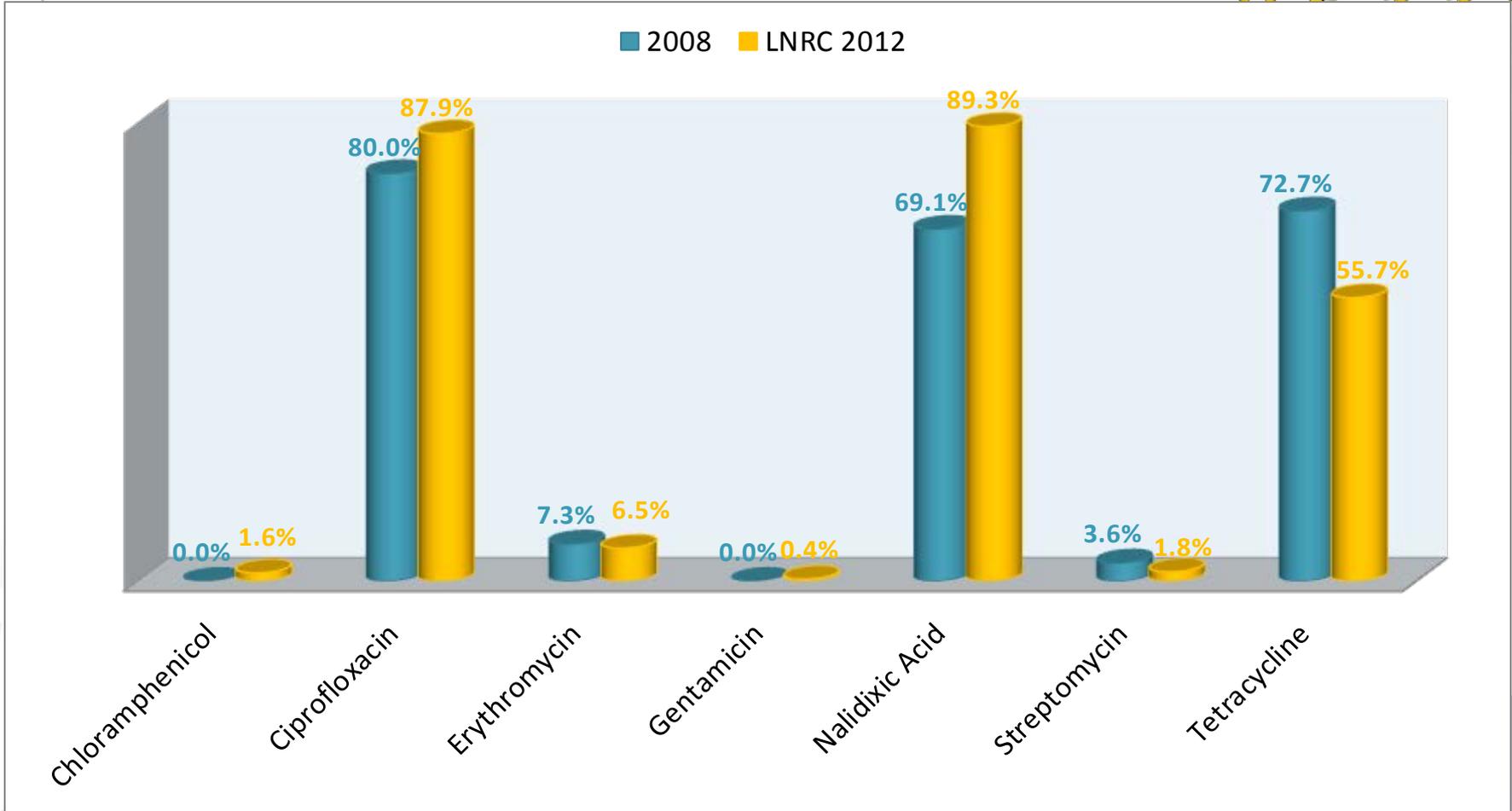


- EFSA-ECDC: The European Union Summary Report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2010. EFSA Journal 2012; 10(3):2598]
- Laboratorio Nazionale Riferimento *Campylobacter* 2012.

# Resistenza in ceppi di *C. jejuni* di origine avicola

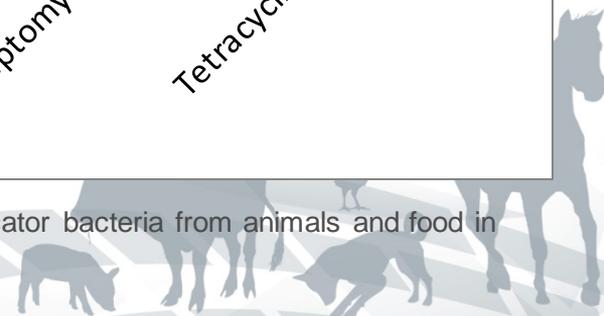


■ 2008 ■ LNRC 2012

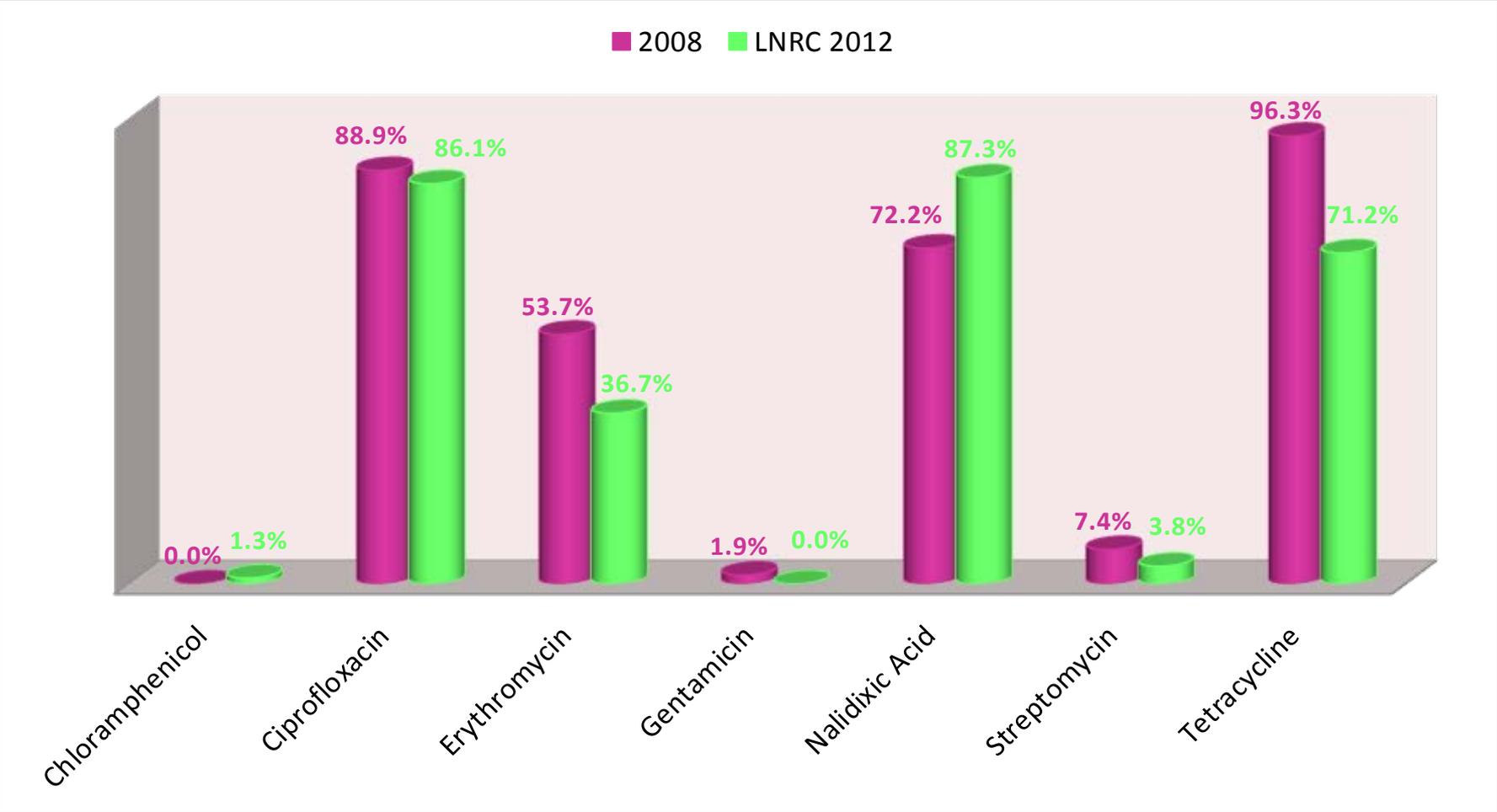
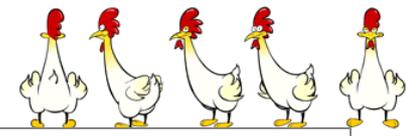


■ EFSA: The Community Summary Report on antimicrobial resistance in zoonotic and indicator bacteria from animals and food in the European Union in 2008. EFSA Journal 2010; 8(7):1658.

■ Laboratorio Nazionale Riferimento *Campylobacter* 2012.

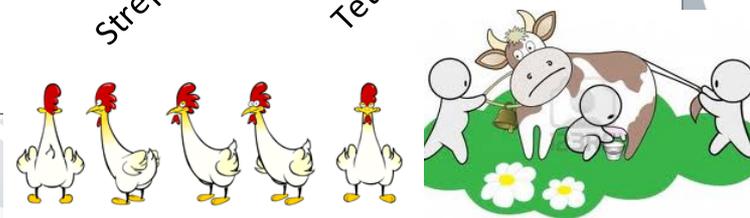
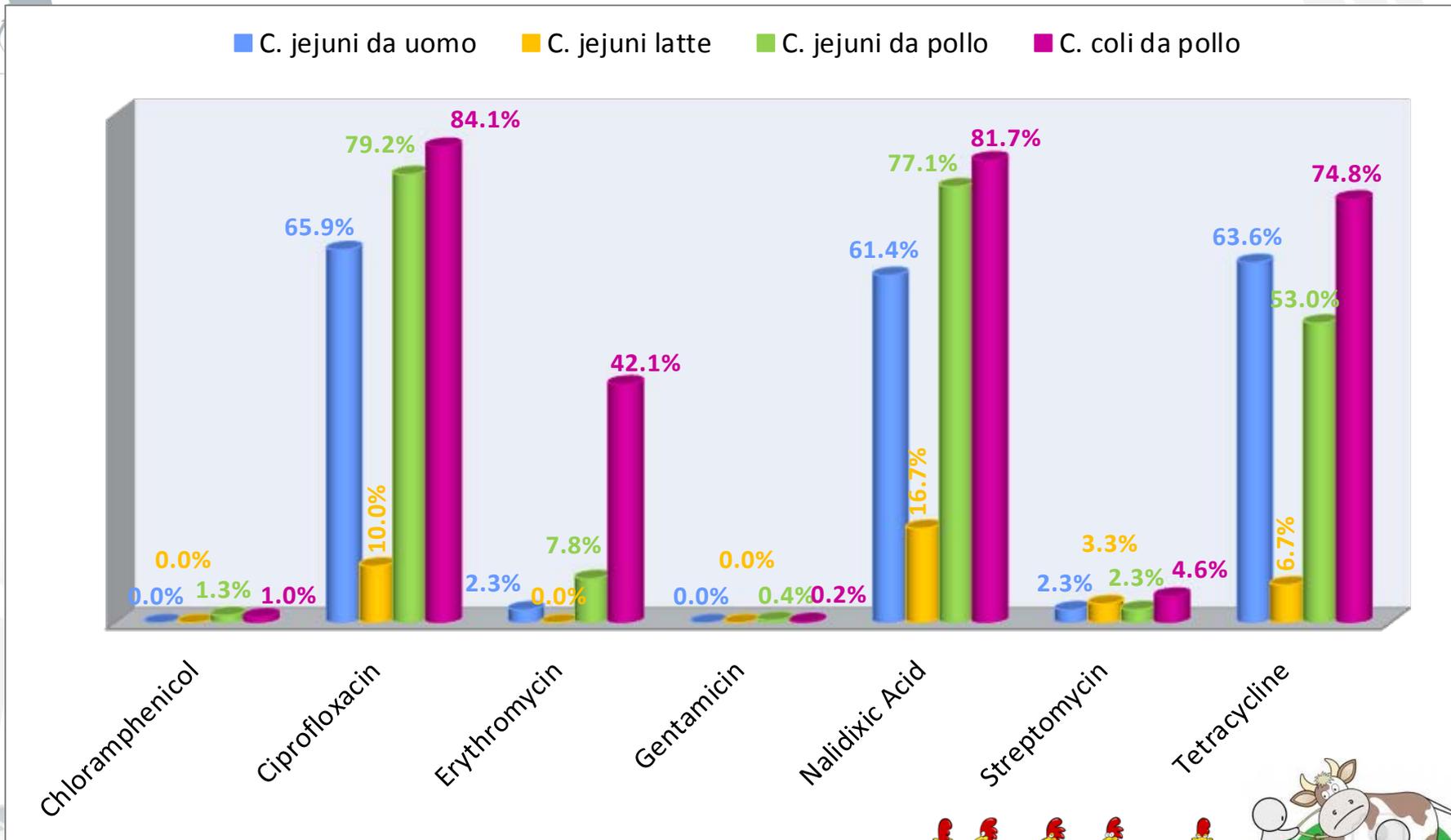


# Resistenza in ceppi di *C. coli* di origine avicola



■ EFSA: The Community Summary Report on antimicrobial resistance in zoonotic and indicator bacteria from animals and food in the European Union in 2008. EFSA Journal 2010; 8(7):1658.  
■ Laboratorio Nazionale Riferimento *Campylobacter* 2012

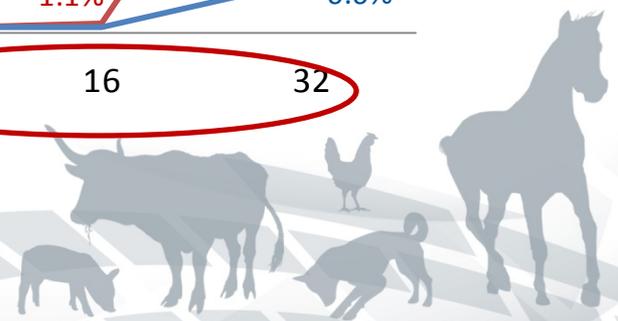
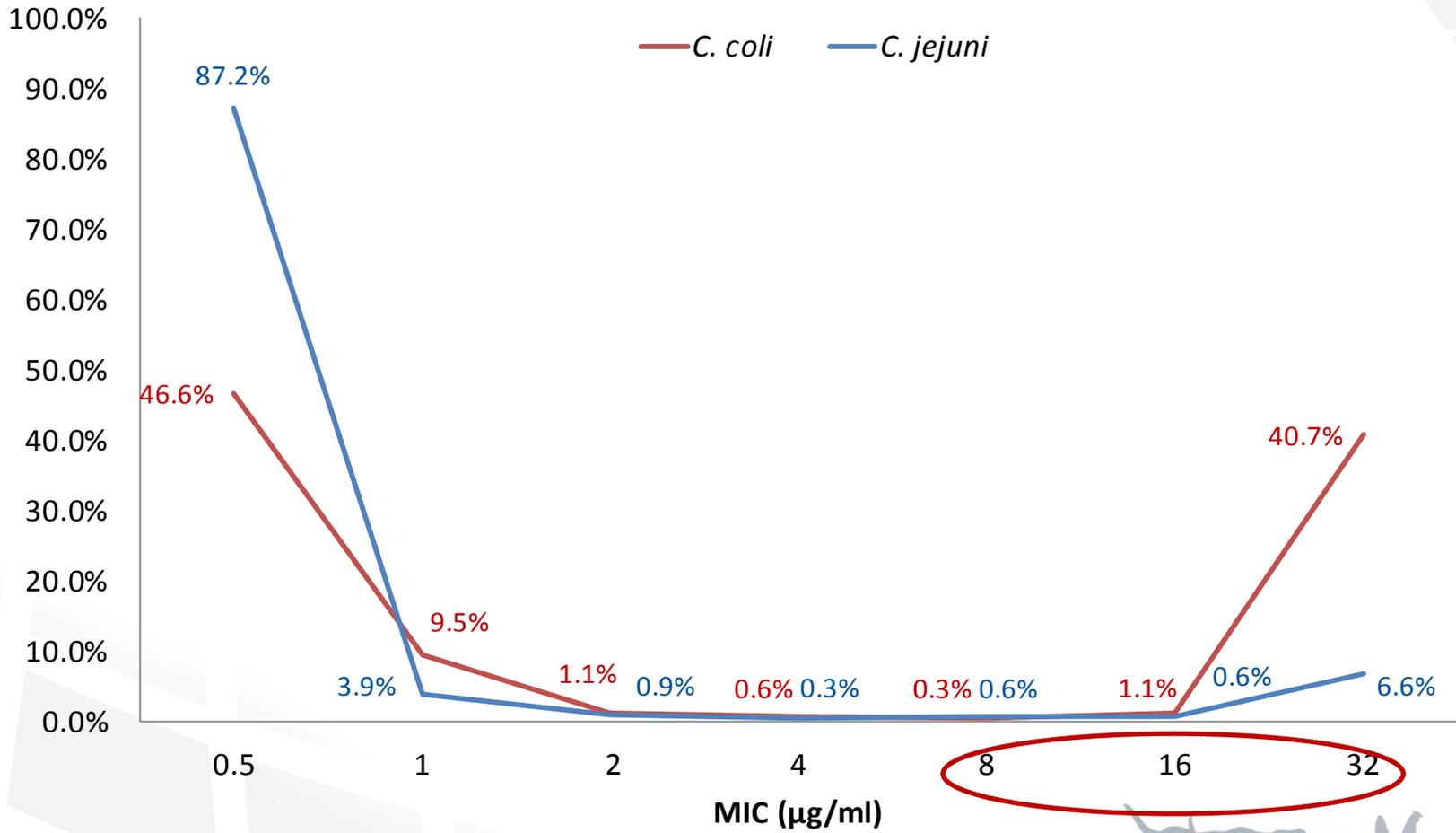
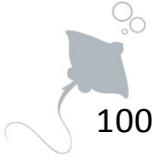
# Resistenza di *Campylobacter* in diverse matrici



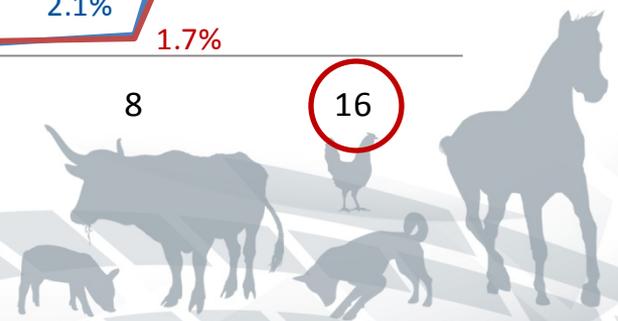
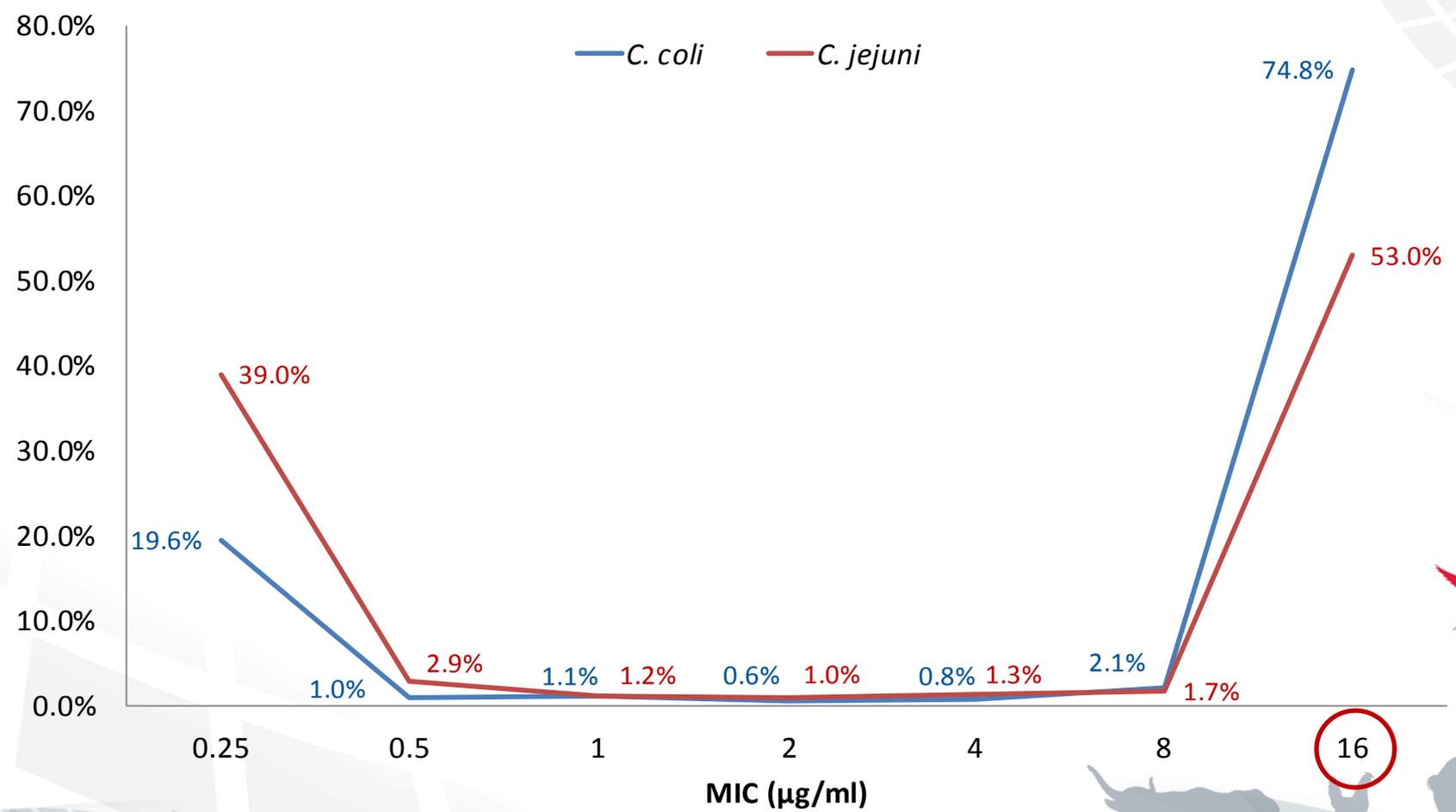


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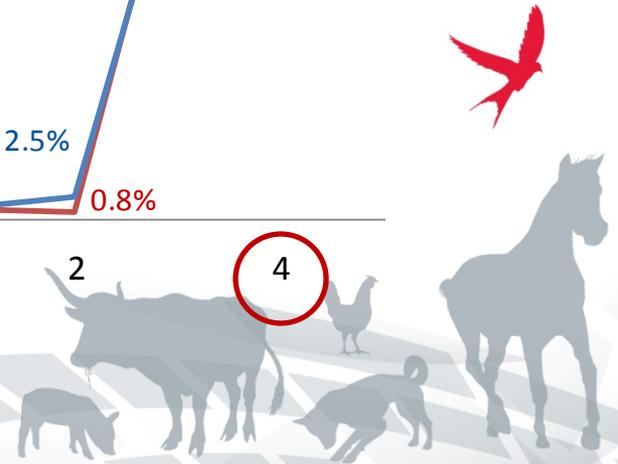
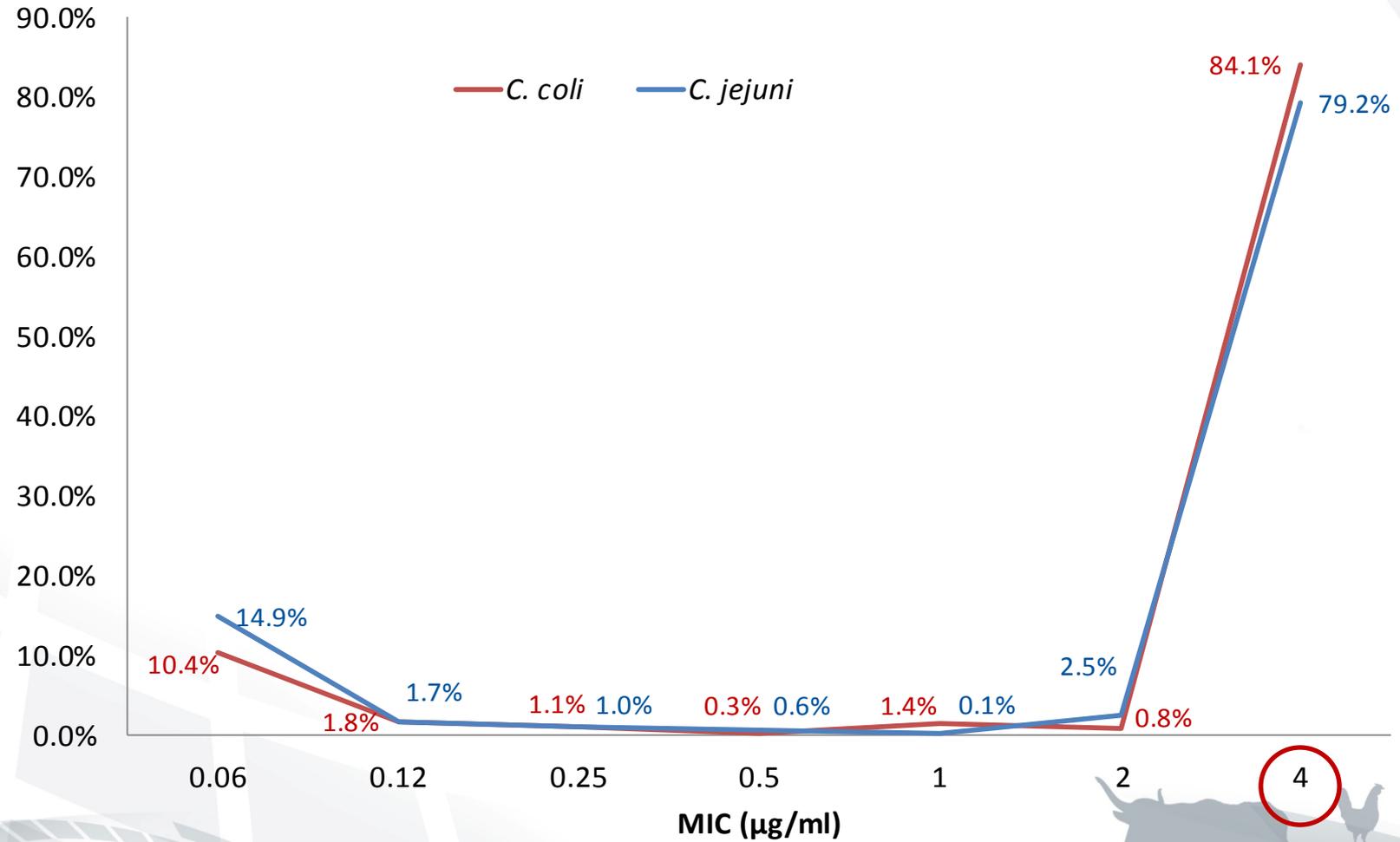
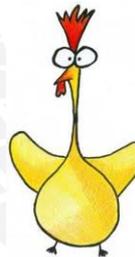
# Distribuzione MIC Eritromicina



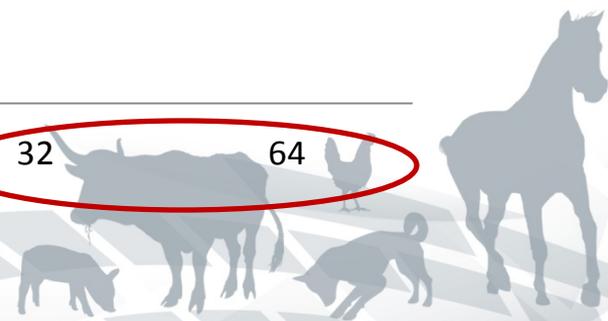
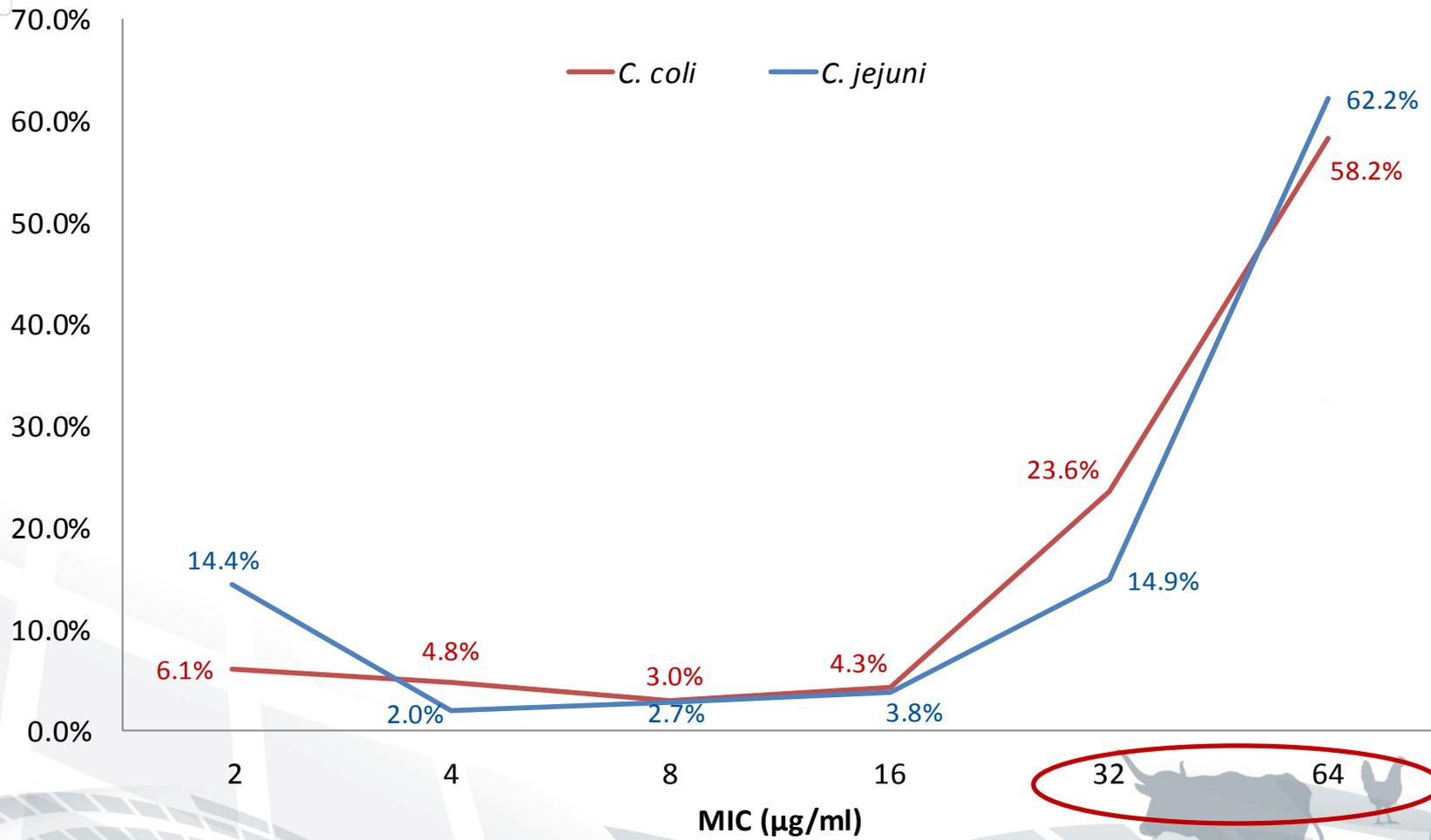
# Distribuzione MIC Tetraciclina



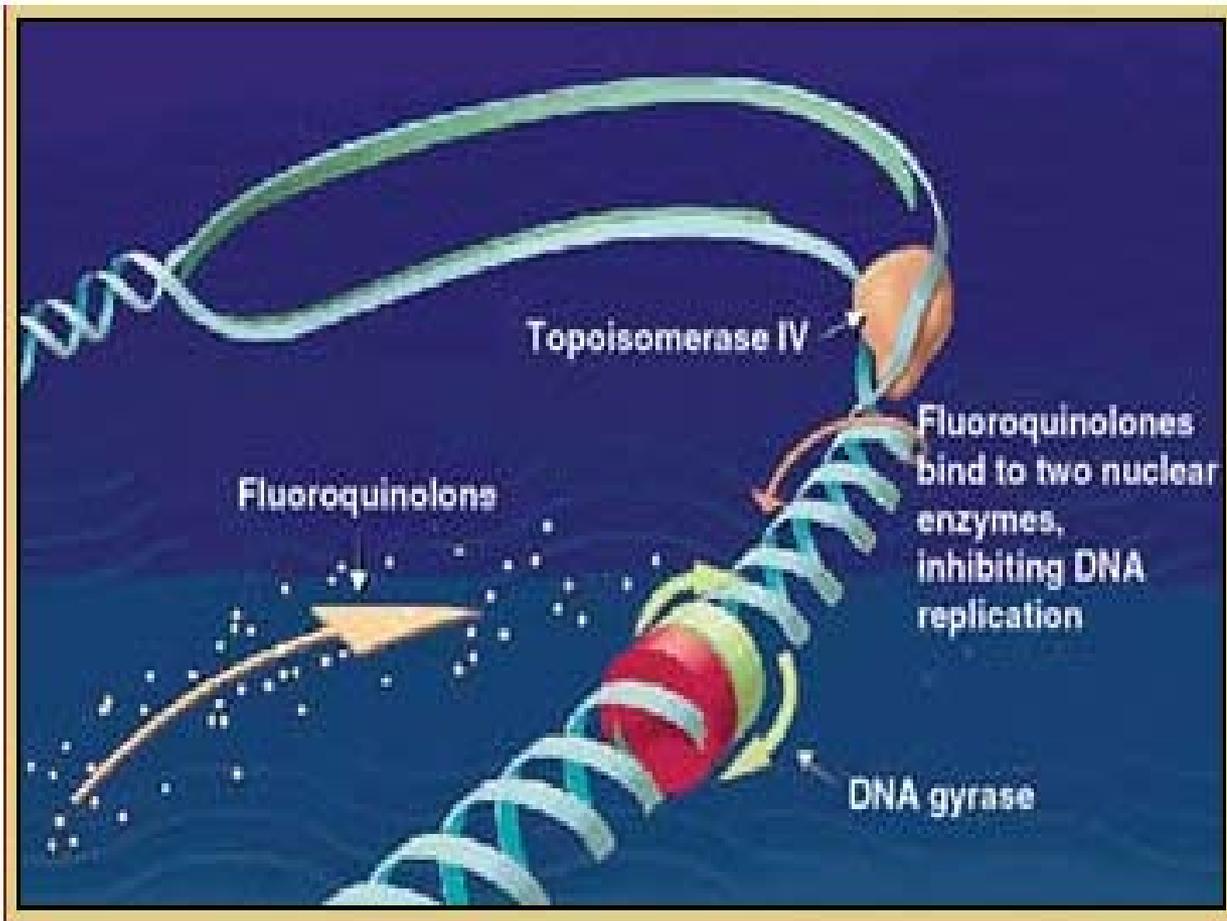
# Distribuzione MIC Ciprofloxacina



# Distribuzione MIC Acido nalidixico



# Resistenza ai chinoloni e fluorochinoloni

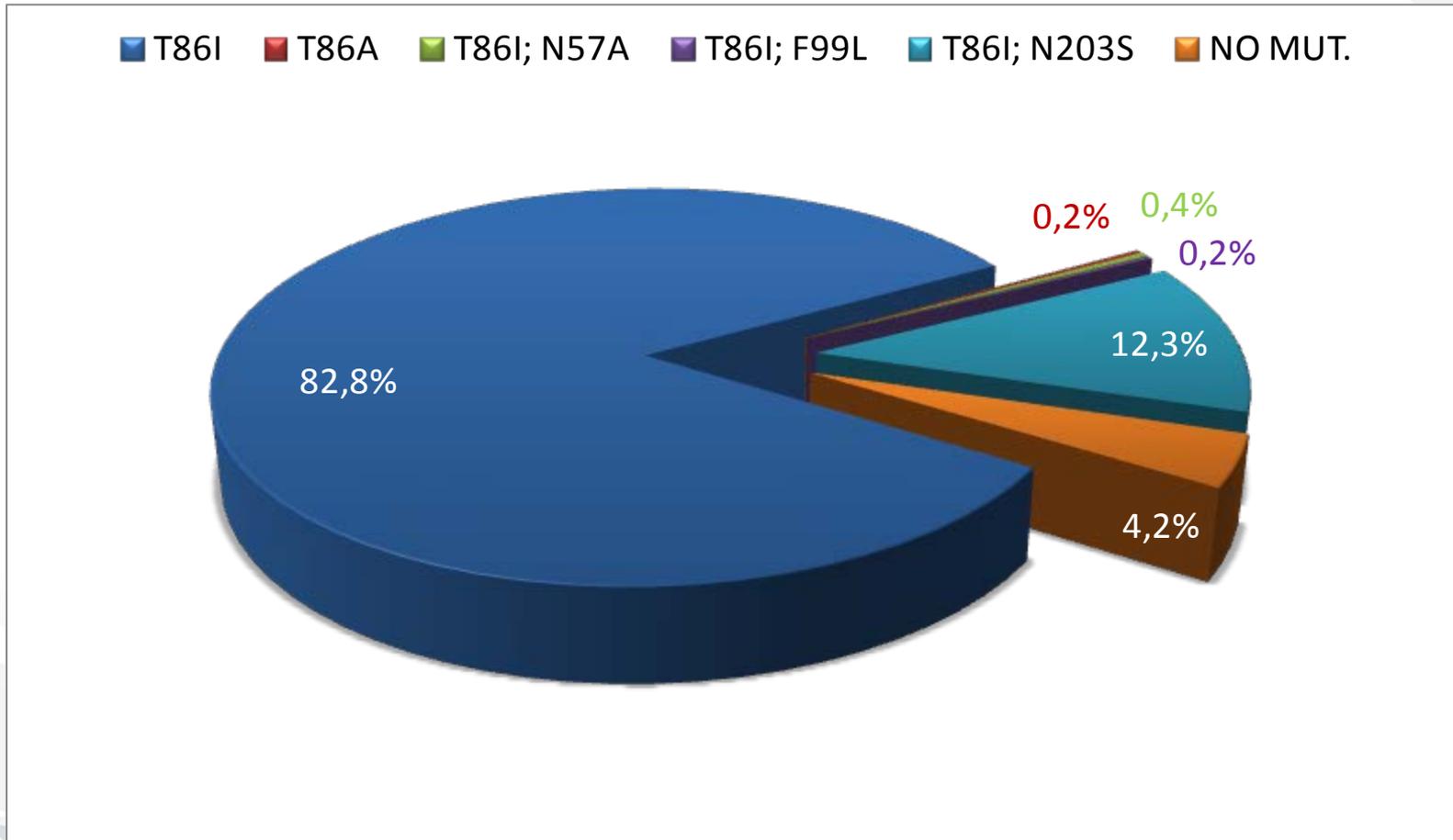


- Sequenziamento di *gyrA* codificante per subunità A della DNA girasi.
- Mutazioni a carico di QRDR (quinolone-resistance-determining region).





# Distribuzione mutazioni *gyrA*



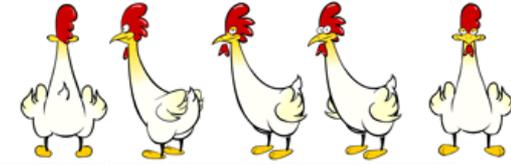
# Multiresistenza

## Fenomeno della multiresistenza in *Campylobacter* non molto diffuso.

- Bywater, R., Deluyker, H., Deroover, E., de Jong, A., Marion, H., McConville, M., Rowan, T., Shryock, T., Shuster, D., Thomas, V., Vallé, M., & Walters, J. (2004). A European survey of antimicrobial susceptibility among zoonotic and commensal bacteria isolated from food-producing animals. *J Antimicrob Chemother*, 54(4), 744-754.
- Miflin, J. K., Templeton, J. M., & Blackall, P. J. (2007). Antibiotic resistance in *Campylobacter jejuni* and *Campylobacter coli* isolated from poultry in the South-East Queensland region. *J Antimicrob Chemother*, 59, 775–778.
- Thorsteinsdottir, T. R., Kristinsson, K. G., Fridriksdottir, V., & Gunnarsson, E. (2008). Antimicrobial Resistance of *Campylobacter* spp. isolated from broiler flocks in Iceland 2001–2005. *Microb Drug Resist*, 14(1), 49-53.
- Aerts M., Jaspers S. Analysis of isolate based data on antimicrobial resistance collected from volunteer Member States for the year 2010. Supporting Publications 2012:EN-308. [204 pp.].



# Multiresistenza in *Campylobacter* di origine avicola



N° antibiotici	Profilo	<i>C. jejuni</i>	<i>C. coli</i>	Percentuale
7	CCipEGNaST	3	0	0,2%
6	CCipENaST	1	2	0,2%
	CipEGNaST	0	1	0,1%
5	CCipENaT	1	2	0,2%
	CENaST	0	3	0,2%
	CipENaST	2	8	0,8%
4	CipENaT	30	164	14,7%
	ENaSTe	0	1	0,1%
	CipNaST	3	3	0,5%
	CcipNaT	3	0	0,2%
	CipEST	0	1	0,1%
<b>TOT ceppi</b>		<b>43</b>	<b>185</b>	<b>17,3%</b>

C: cloramfenicolo; Cip: ciprofloxacina; E: eritromicina; G: gentamicina; Na: acido nalidixico; S: streptomina; T: tetraciclina  
Dati su 1317 ceppi esaminati.

# Direttive EFSA

Technical specifications on the harmonised monitoring and reporting of antimicrobial resistance in *Salmonella*, *Campylobacter* and indicator *Escherichia coli* and *Enterococcus spp.* bacteria transmitted through food. *EFSA Journal* 2012; 10(6):2742.

- Utilizzo del metodo della microdiluizione.
- Utilizzo dei breakpoint clinici per interpretare i dati umani.
- Utilizzo dei cut-off epidemiologici proposti dall'EUCAST per determinare la resistenza microbiologica.
- Il range di concentrazione degli antibiotici testati deve coprire sia i cut-off epidemiologici che i breakpoint.



# MIC Breakpoints ( $\mu\text{g/ml}$ )

Antimicrobial	Species	CLSI Values ( $\mu\text{g/ml}$ )	EUCAST Values ( $\mu\text{g/ml}$ )		Range of concentration ( $\mu\text{g/ml}$ )		
		Resistant breakpoint	ECOFF	Clinical resistance breakpoint	Current recommendation*	New recommendation*	EUCAMP Panel <sup>o</sup>
Chloramphenicol	<i>C. jejuni</i>	>16	>16	ND			2-32
	<i>C. coli</i>	>16	>16	ND			
Ciprofloxacin	<i>C. jejuni</i>	>2	> 0.5	> 0.5	0.06-8	0.06-32	0.06-4
	<i>C. coli</i>	>2	> 0.5	> 0.5			
Erythromycin	<i>C. jejuni</i>	>4	>4	>4	0.5-64	0.25-128	0.5-32
	<i>C. coli</i>	>4	>8	>8			
Gentamicin	<i>C. jejuni</i>	>8	>2	ND	0.12-16	0.12-16	0.12-16
	<i>C. coli</i>	>8	>2	ND			
Nalidixic Acid	<i>C. jejuni</i>	>16	>16	ND			2-64
	<i>C. coli</i>	>16	>16	ND			
Streptomycin	<i>C. jejuni</i>	>4	>4	ND	0.5-32	0.5-256	1-16
	<i>C. coli</i>	>4	>4	ND			
Tetracycline	<i>C. jejuni</i>	>8	>1	>2	0.12-16	0.25-128	0.25-16
	<i>C. coli</i>	>8	>2	>2			

**CLSI:** Clinical and Laboratory Standards Institute. 2003. Performance Standard for Antimicrobial Susceptibility Testing; Vol. M100-S13 Table 2A.

**Eucast:** European Committee on Antimicrobial Susceptibility Testing. 2012. Clinical breakpoints, epidemiological cut-off (ECOFF) values and EUCAST disk diffusion methodology for *Campylobacter jejuni* and *Campylobacter coli*.

\*:EFSA-ECDC; Technical specifications on the harmonised monitoring and reporting of antimicrobial resistance in *Salmonella*, *Campylobacter* and indicator *Escherichia coli* and *Enterococcus spp. bacteria transmitted through food*. *EFSA Journal* 2012; 10(6):2742.

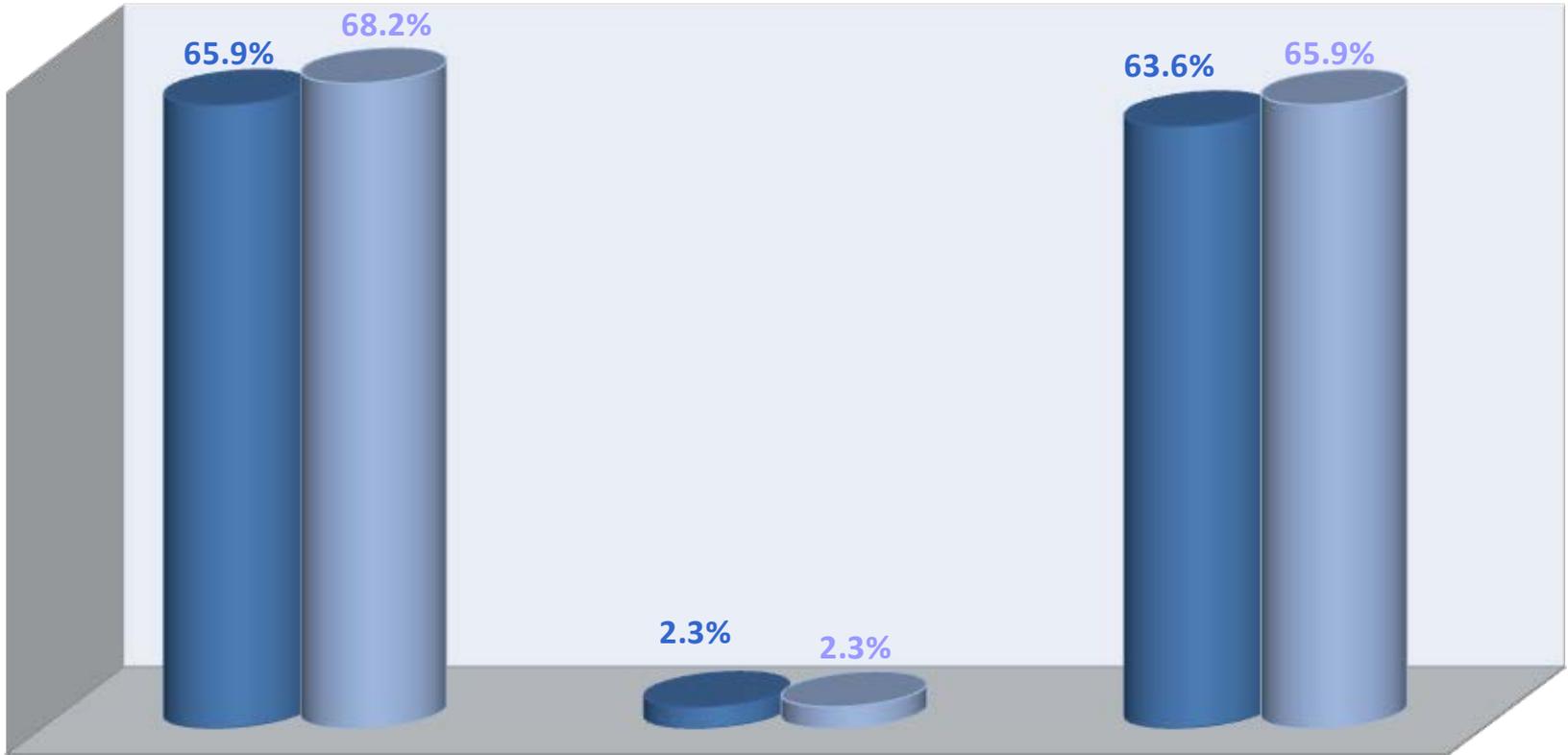
<sup>o</sup>EUCAMP Panel (Sensititre system, Trek Diagnostic)

**ND:** non determinato.

# *C. jejuni* di origine umana



■ CLSI   ■ Eucast



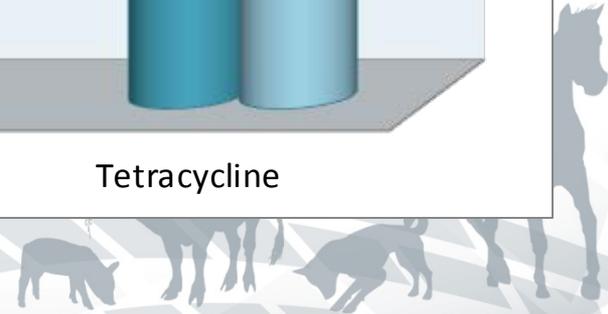
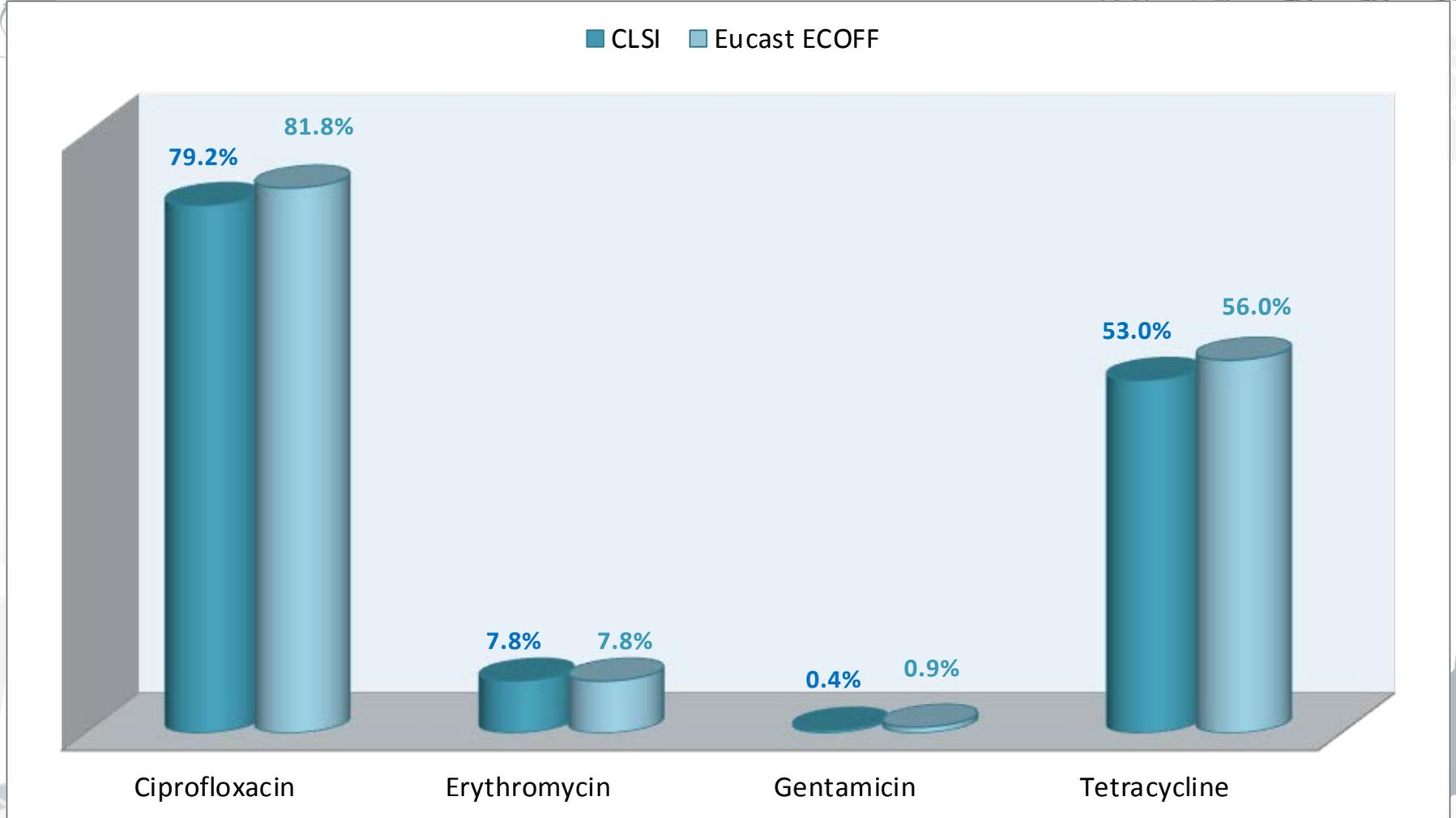
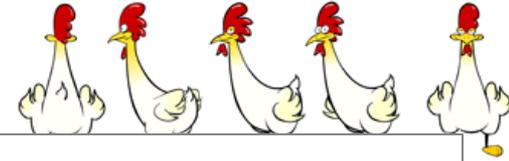
Ciprofloxacin

Erythromycin

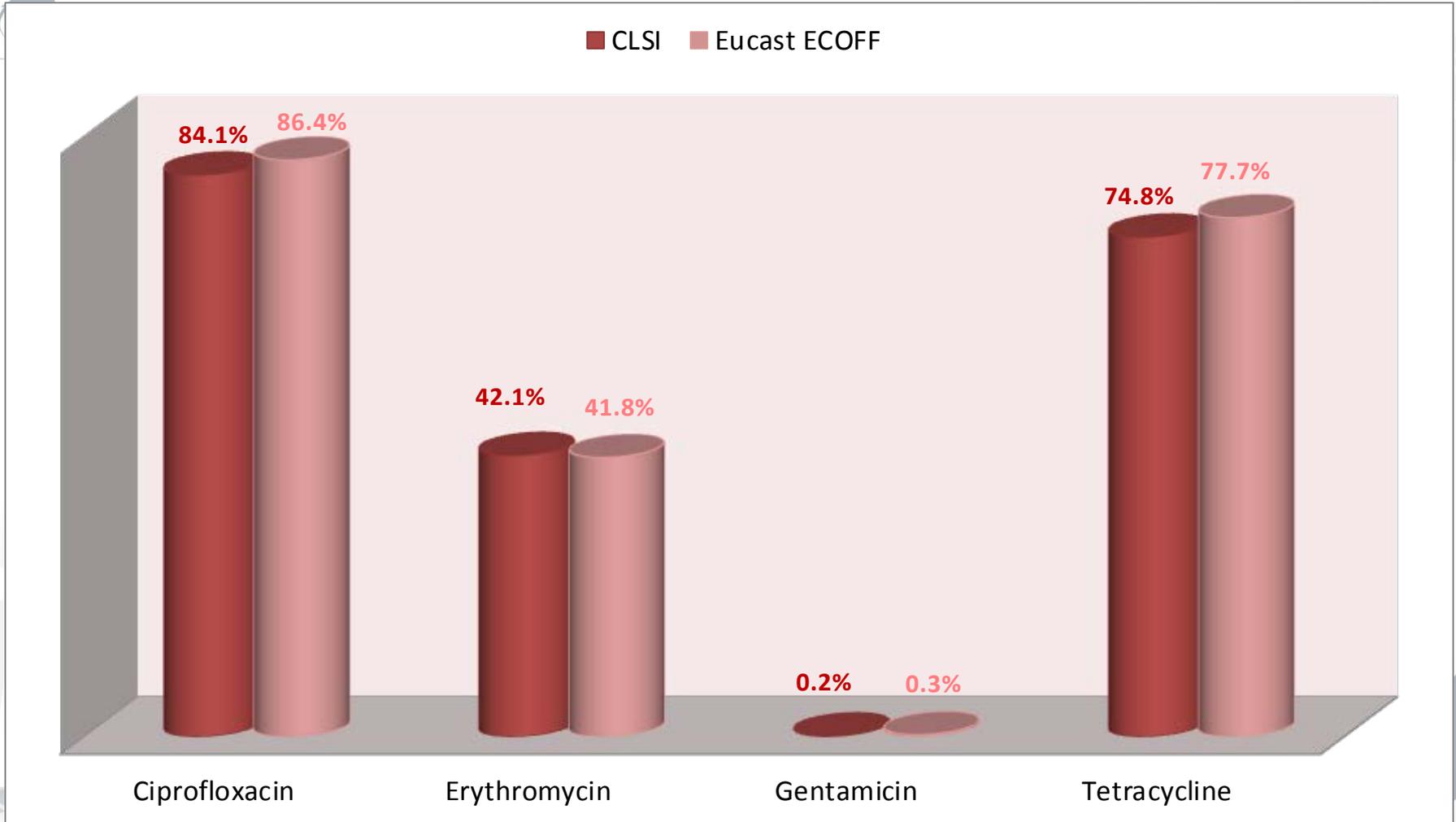
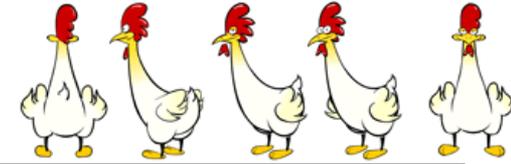
Tetracycline



# *C. jejuni* di origine avicola



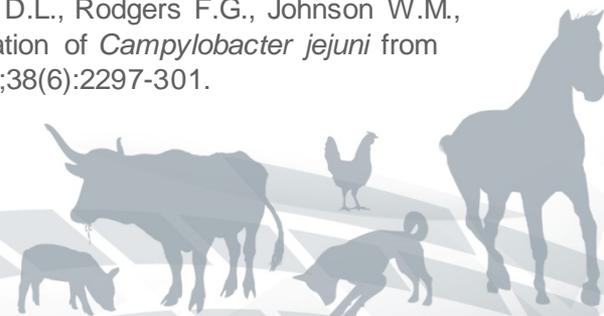
# *C. coli* di origine avicola



# Sierotipizzazione di *C. jejuni*

- Non sono disponibili dati recenti.
- Possibile correlazione tra sierotipo e sviluppo di neuropatie autoimmuni in seguito a complicazioni post-infettive.

- Islam Z., Van Belkum A., Cody A.J., Tabor H., Jacobs B.C., Talukder K.A., Endtz H.P.. (2009) *Campylobacter jejuni* HS:23 and Guillain-Barré Syndrome, Bangladesh. *Emerging Infection Diseases* Vol. 15 (8):1315-1317
- Yan S.S., Pendrak M.L., Foley S.L., Powers J.H. 2005. *Campylobacter* infection and Guillain-Barre´ syndrome: public health concerns from a microbial food safety perspective. *Clinical and Applied Immunology, Reviews* 5, p.285–305.
- Endtz H.P., Ang C.W., van Den Braak N., Duim B., Rigter A., Price L.J., Woodward D.L., Rodgers F.G., Johnson W.M., Wagenaar J.A., Jacobs B.C., Verbrugh H.A., van Belkum A. Molecular characterization of *Campylobacter jejuni* from patients with Guillain-Barré and Miller Fisher syndromes. *J Clin Microbiol.* 2000 Jun;38(6):2297-301.



# Distribuzione sierotipi *C. jejuni*

Gruppo: HS	%	Gruppo: HS	%	Gruppo: HS	%
A: 1,44	11,3%	K:12	0,3%	Y: 37	2,3%
B: 2	2,7%	L: 15	11,0%	Z: 38	0,0%
C: 3	8,8%	N: 18	0,0%	Z <sub>2</sub> : 41	0,0%
D: 4,13,16,43,50	11,5%	O: 19	0,1%	Z <sub>4</sub> : 45	0,1%
E: 5	0,5%	P: 21	0,3%	Z <sub>5</sub> : 52	7,6%
F: 6,7	8,4%	R: 23,36,53	0,7%	Z <sub>6</sub> : 55	4,7%
G: 8	0,4%	S: 27	0,3%	Z <sub>7</sub> : 57	0,4%
I: 10	0,1%	U: 31	0,1%	NC	5,6%
J: 11	2,0%	V: 32	0,4%	NT	19,6%

NC: non classificabili  
NT: non tipizzabili  
Dati su 741 ceppi esaminati.

# Sierotipizzazione di *C. jejuni*

- La presenza di molti ceppi non tipizzabili o non classificabili è molto comune.

- Eyles, R. F., Brooks, H. J. L., Townsend, C. R., Burtenshaw, G. A., Heng, N. C. K., Jack, R. W., & Weinstein, P. (2006). Comparison of *Campylobacter jejuni* PFGE and Penner subtypes in human infections and in water samples from the Taieri River catchment of New Zealand. *J Appl Microbiol*, 101, 18–25.
- Boonmar, S., Sangsuk, L., Suthivarakom, K., Padungtod, P. & Morita, Y. (2005). Serotypes and antimicrobial resistance of *Campylobacter jejuni* isolated from humans and animals in Thailand. *Southeast Asian J Trop Med Public Health*, 36, 130-134.
- Nielsen, E. M., Engberg, J., & Madsen, M. (1997). Distribution of serotypes of *Campylobacter jejuni* and *C. coli* from Danish patients, poultry, cattle and swine. *FEMS Immunol Med Microbiol*, 19, 47-56.
- Nielsen, E. M., Fussing, V., Engberg, J., Nielsen, N. L., & Neimann, J. (2006). Most *Campylobacter* subtypes from sporadic infections can be found in retail poultry products and food animals. *Epidemiol Infect*, 134, 758–767.

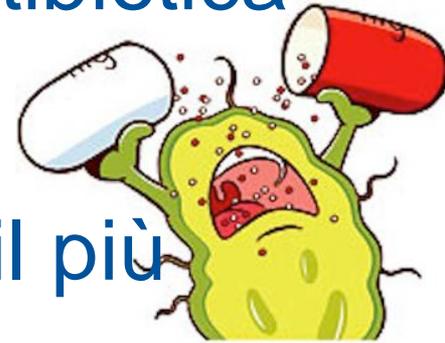
- *Campylobacter* è fortemente sensibile a condizioni di stress (isolamento, congelamento).

- Nakari, U. M., Laaksonen, K., Korkeila, M., & Siitonen, A. (2005). Comparative typing of *Campylobacter jejuni* by heat-stable serotyping and PCR-based restriction fragment length polymorphism analysis. *J Clin Microbiol*, 43, 1166-1170.
- Oza, A. N., Thwaites, R. T., Wareing, D. R., Bolton, F. J., & Frost, J. A. (2002). Detection of heat-stable antigens of *Campylobacter jejuni* and *C. coli* by direct agglutination and passive hemagglutination. *J Clin Microbiol* 40, 996-1000.



## ... per concludere

- Conferma dell'elevata resistenza antibiotica ed aumento dei ceppi multiresistenti.
- Necessità di un uso consapevole e il più possibile limitato degli antibiotici.
- Uso mirato delle molecole → antibiogramma.
- Piano di azione europeo sulla resistenza agli antibiotici 2011-2015.



# Ringraziamenti



Federico  
Di Fabio

Giuliano  
Garofolo

Antonietta  
Pomanti

GRAZIE PER  
L'ATTENZIONE!

Tiziana  
Persiani

Francesca  
Marotta

Silvana  
Salvatore

Alessandra  
Alessiani

Katuscia  
Zilli

Dr.ssa  
Elisabetta  
Di Giannatale

Gabriella  
Di Serafino