

# Efflux Pumps & Gyrase A Gene Mutation on Fluoroquinolone Resistance in *Campylobacter jejuni/coli*

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# Foodborne Bacterial Infections in the U.S.

Microorganisms	Estimated cases	% Foodborne
<i>Campylobacter</i>	2,453,926	80
<i>Salmonella</i>	1,413,322	95
<i>Shigella</i>	448,240	20
<i>Escherichia coli</i>	269,060	80
<i>Yersinia enterocolitica</i>	96,368	90
<i>Vibrio</i>	8,028	65
<i>Listeria monocytogenes</i>	2,518	99

Mead et al, 1999 *Emerging Infectious Diseases*.

# Drugs of Choice for Campylobacteriosis

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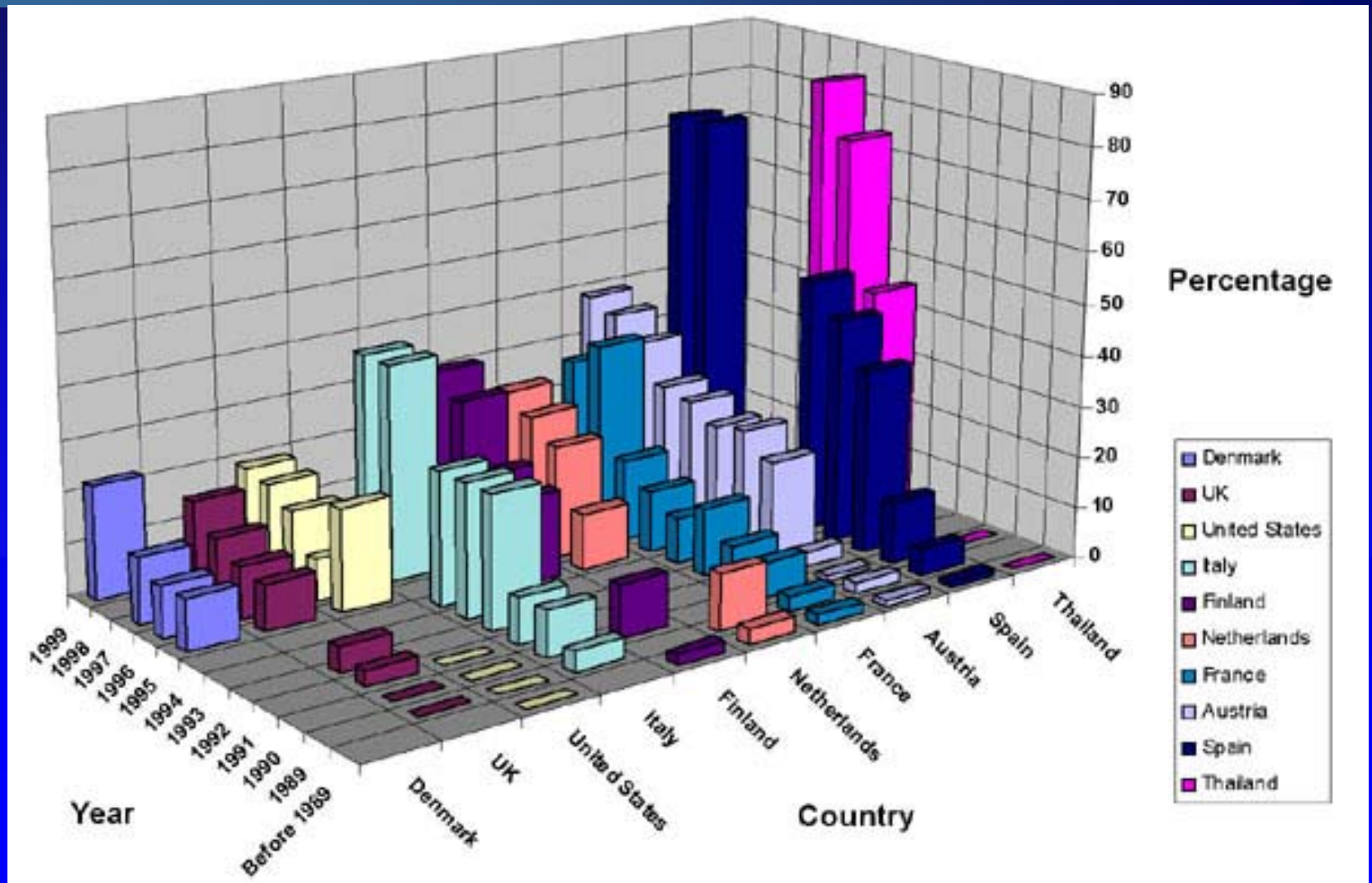


Erythromycin  
Inhibit protein synthesis

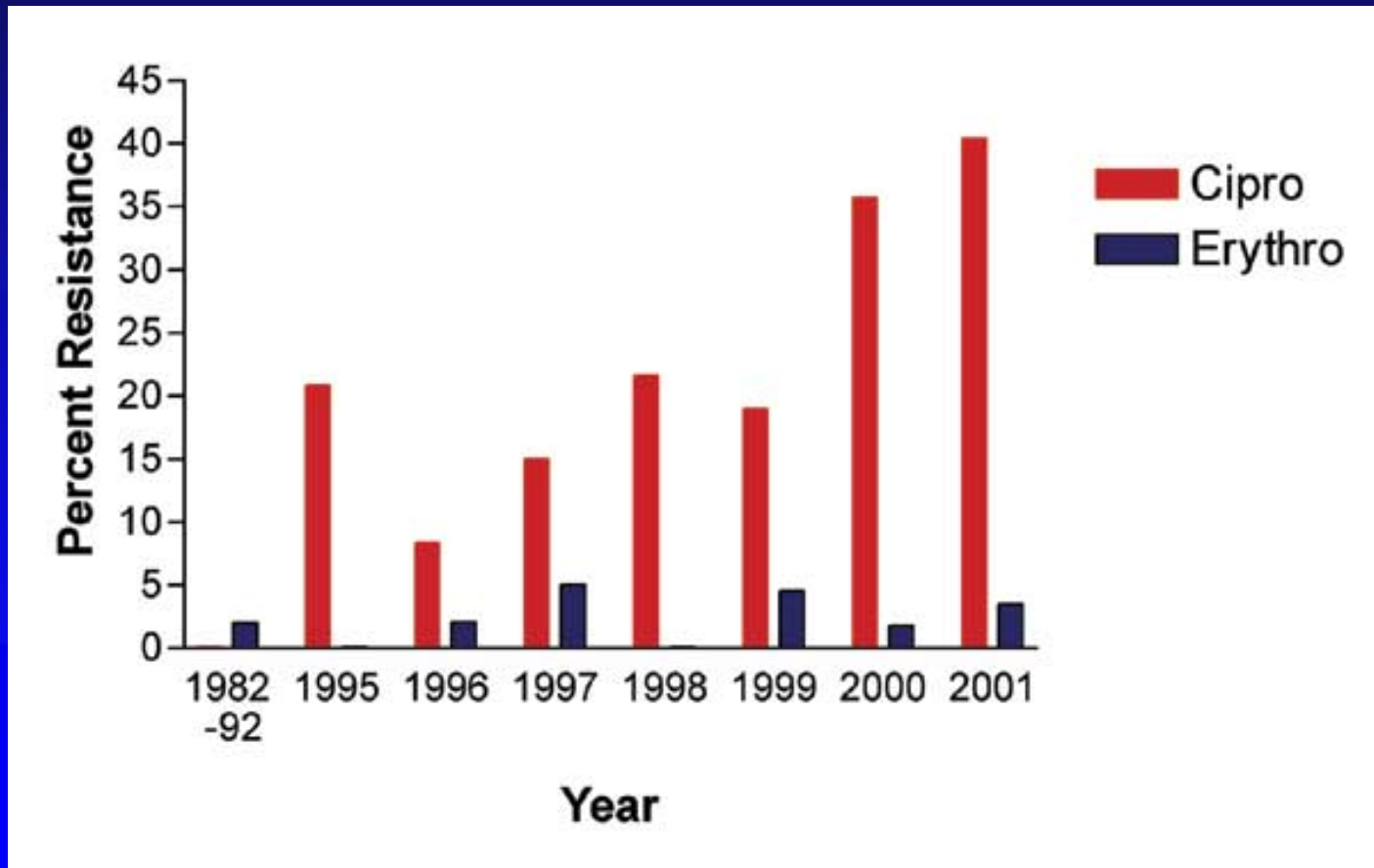


Ciprofloxacin  
Inhibit DNA replication

# Trends in Fluoroquinolone Resistance in *Campylobacter*



# Trends in Erythromycin and Ciprofloxacin Resistance in *C. jejuni*. Philadelphia, USA



Nachamkin, et al. 2002, EID

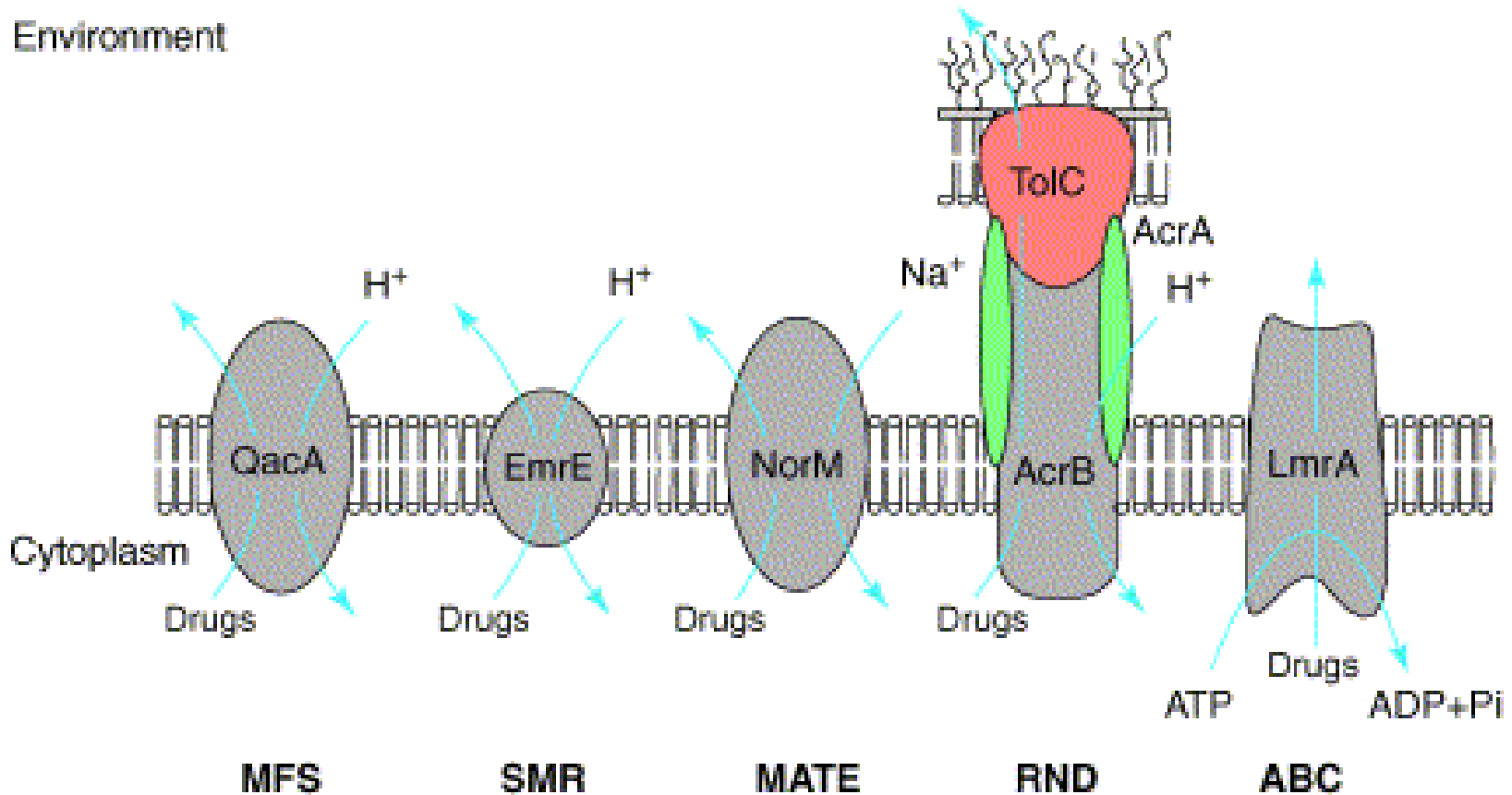
# Mechanisms of Erythromycin and Ciprofloxacin Resistance

	Target alteration	Efflux pumps
Ery	Point mutations in Domain V of 23S rRNA	Over expression
Cip	Point mutations in <i>gyrA</i>	Over expression

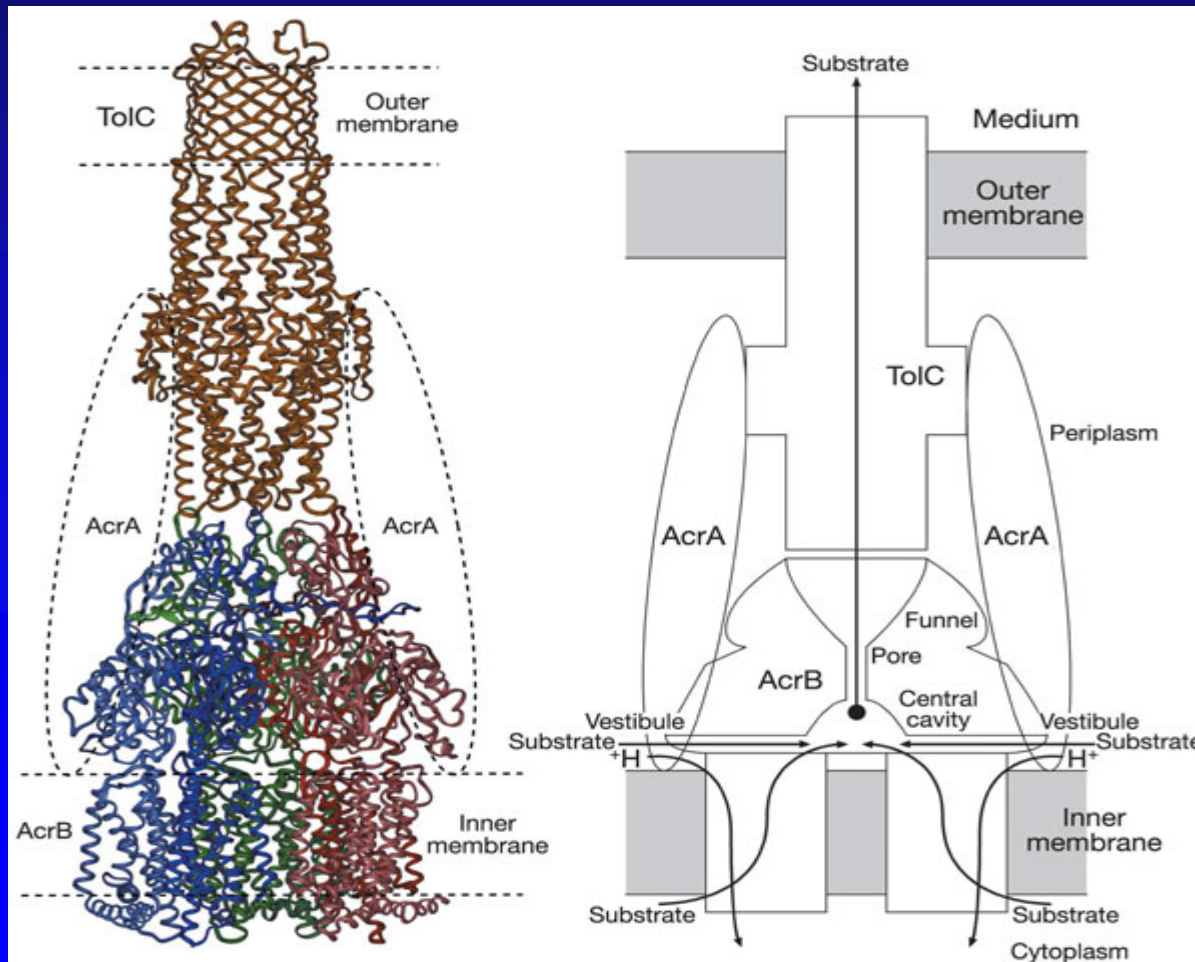
\*\*Efflux pumps are membrane proteins that extrude toxic substances including antibiotics

# Multidrug Efflux Pumps Families

Environment



# AcrA, AcrB, and TolC Efflux Pump Complex





# Role of Efflux Pumps on Antimicrobial Resistance

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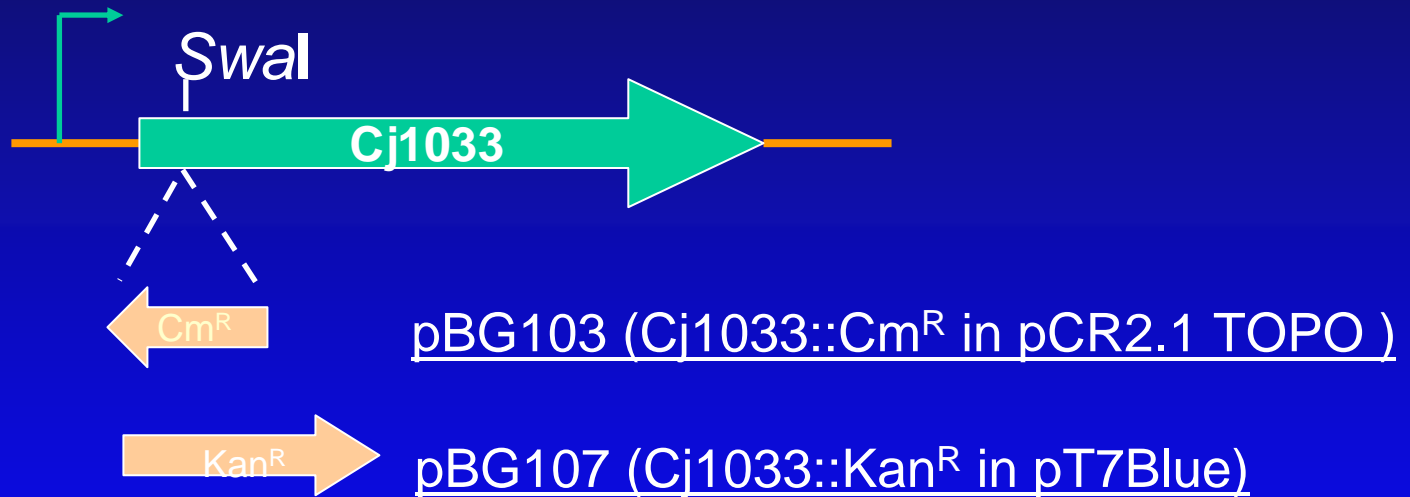
- Identify efflux pumps based bioinformatics database
- Inactivate efflux pump genes
- Determine functions

# Putative Efflux Pump Genes in *C. jejuni*

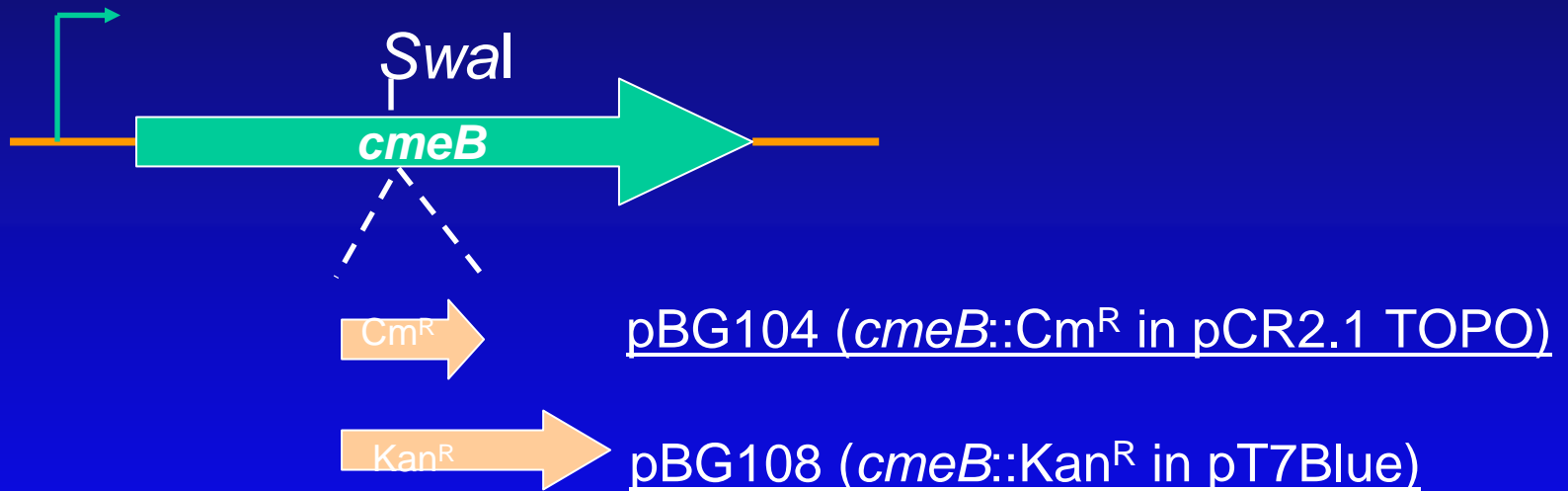
Gene / gene cluster	Family	Sizes (bp)
<i>Cj0035c</i>	MFS	1203
<i>Cj0309c, Cj0310c</i>	DMT	315, 339
<i>Cj0365c (cmeC), Cj0366c (cmeB)</i> <i>Cj0367c (cmeA)</i>	RND	1479, 3123, 1104
<i>Cj0560</i>	MATE	1329
<i>Cj0619</i>	MATE	1317
<i>Cj1031, Cj1032, Cj1033</i>	RND	1275, 741, 3018
<i>Cj1173, Cj1174</i>	DMT	342, 309
<i>Cj1241</i>	MFS	1200
<i>Cj1257c</i>	MFS	1185
<i>Cj1687</i>	MFS	1272



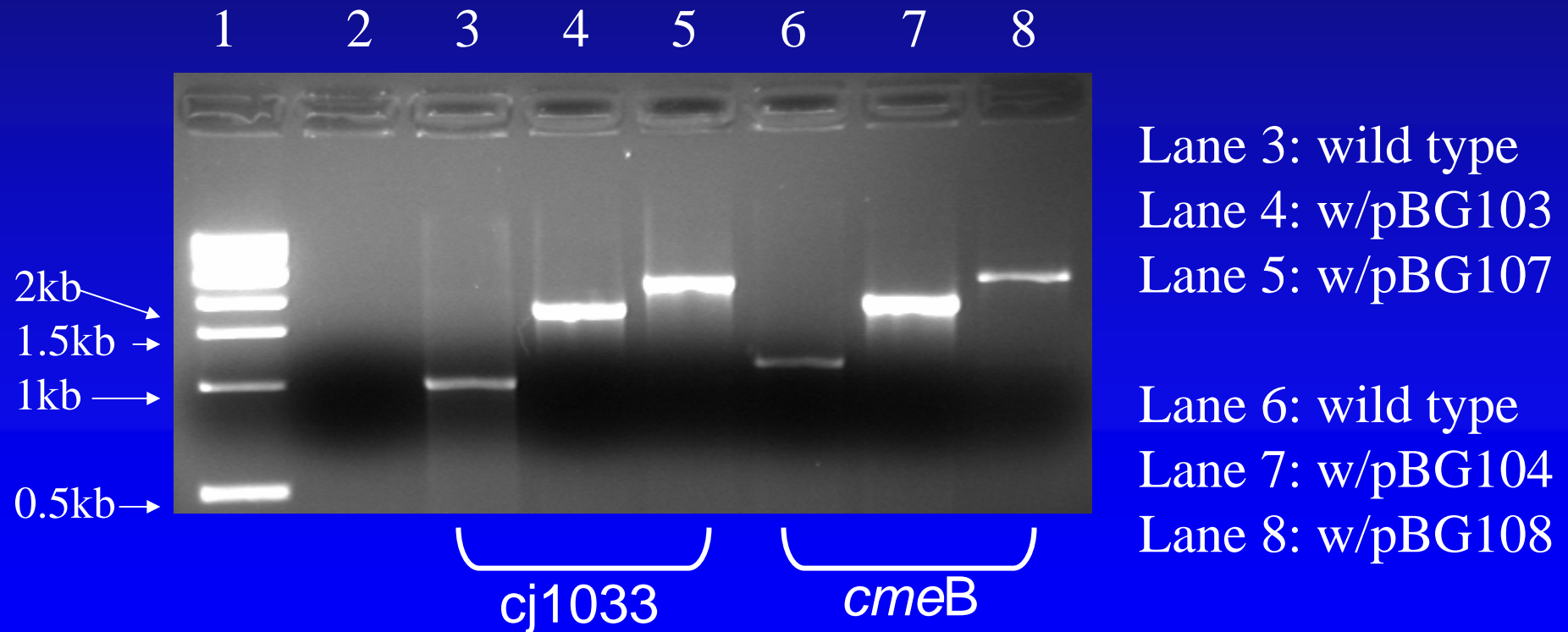
# Cj1033 Construct



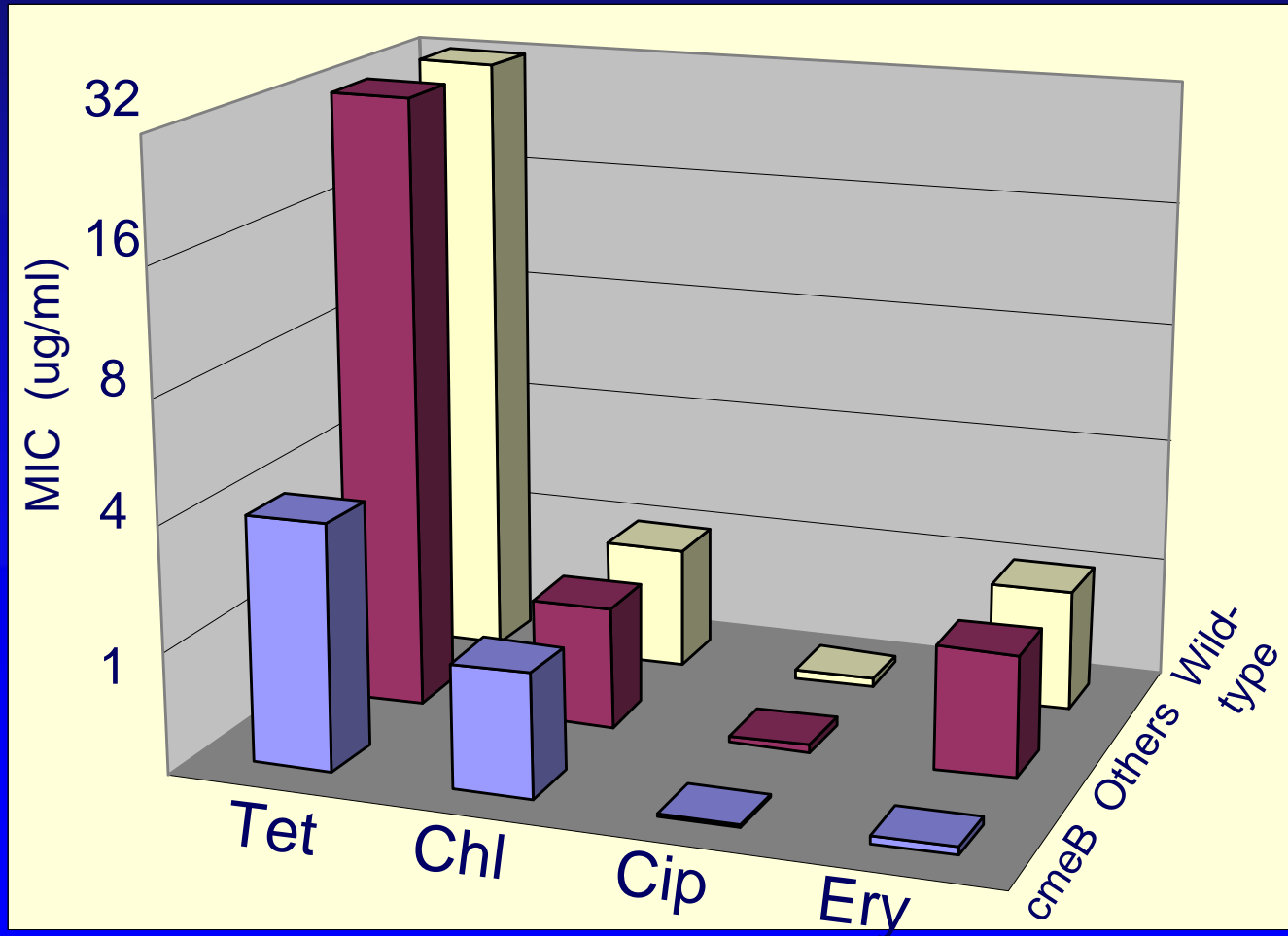
# *cmeB* (Cj0366c) Construct



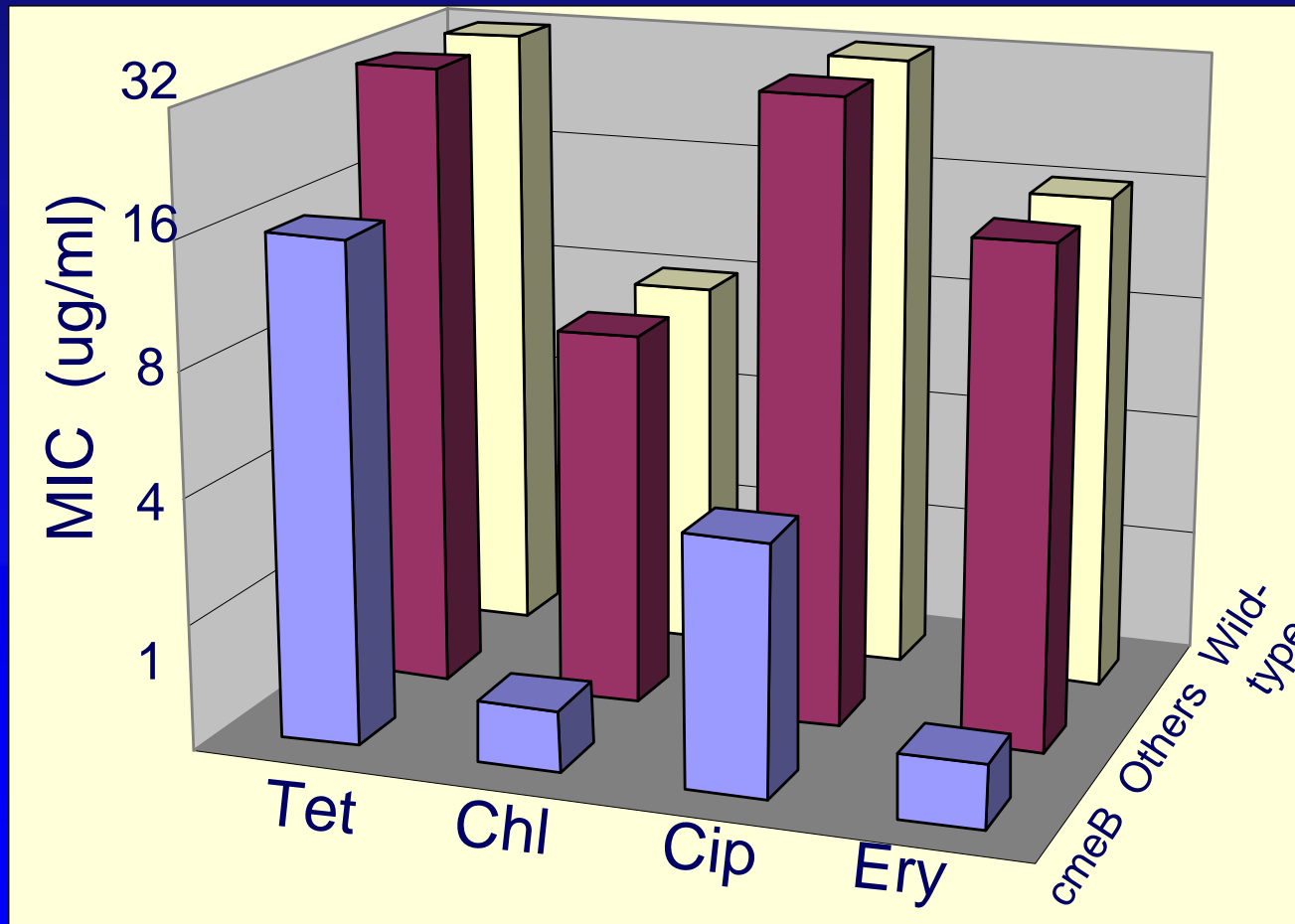
# Confirmation of Insertional Mutation



# Antibiotic Minimum Inhibitory Concentration (MIC) of 81-176 and its Mutants



# MIC of *C. coli* Strain 124 and Its Mutants



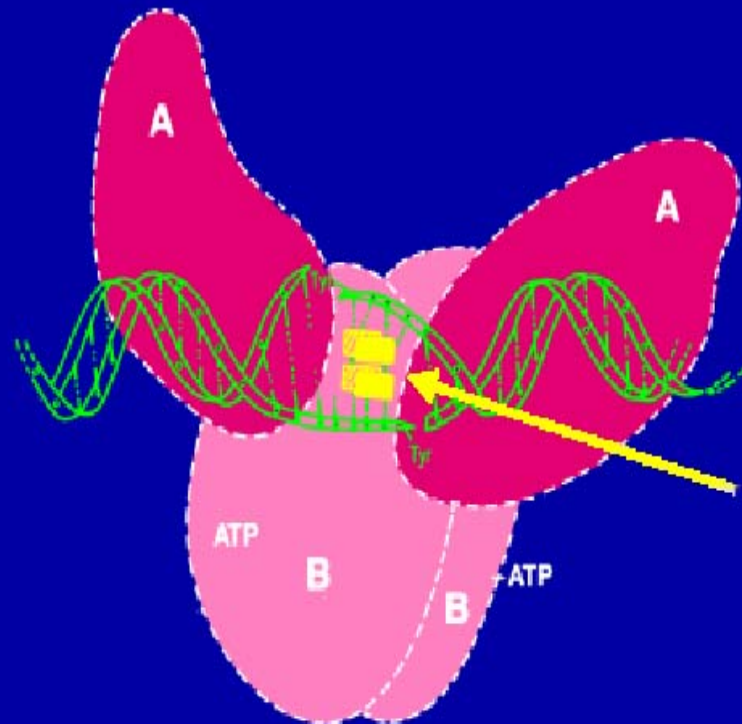


# Mechanisms of Erythromycin and Ciprofloxacin Resistance

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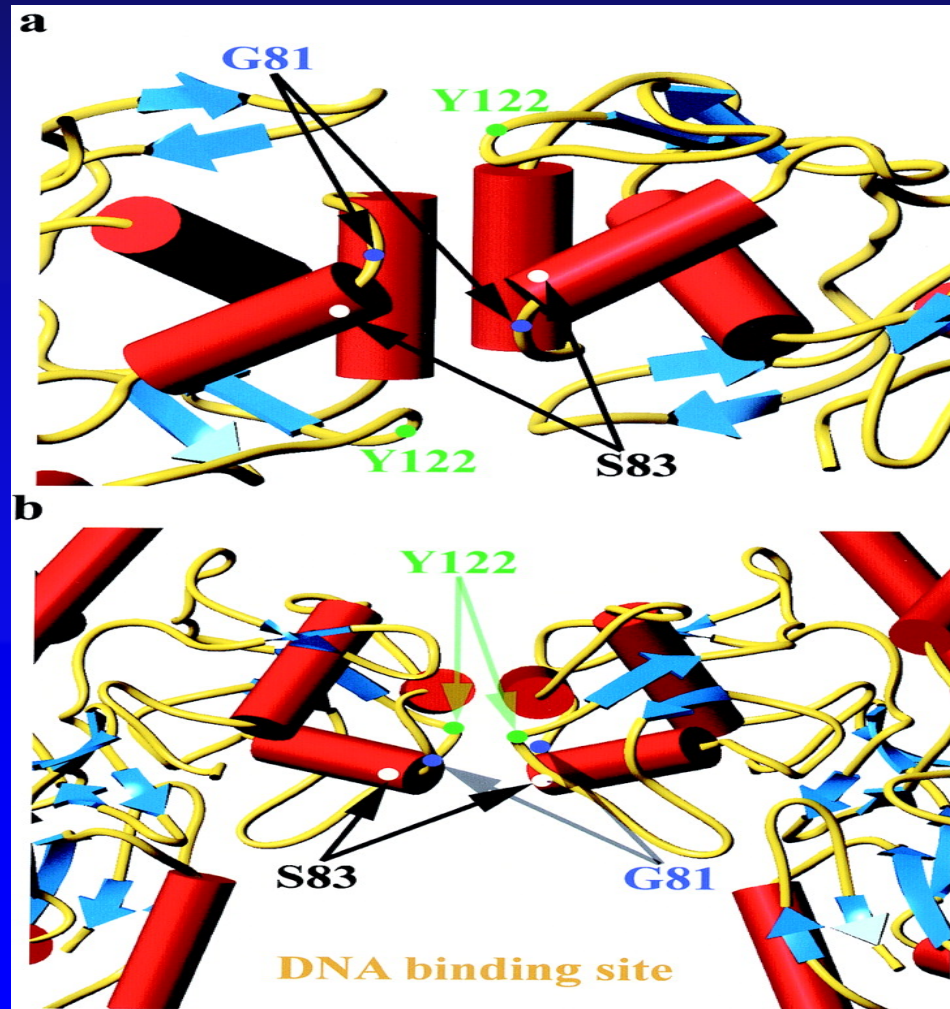
\*\*Efflux pumps are membrane proteins that extrude toxic substances including antibiotics

# How Fluoroquinolone Drugs Work



Inhibit DNA replication

# Amino Acid Substitutions in GyrA

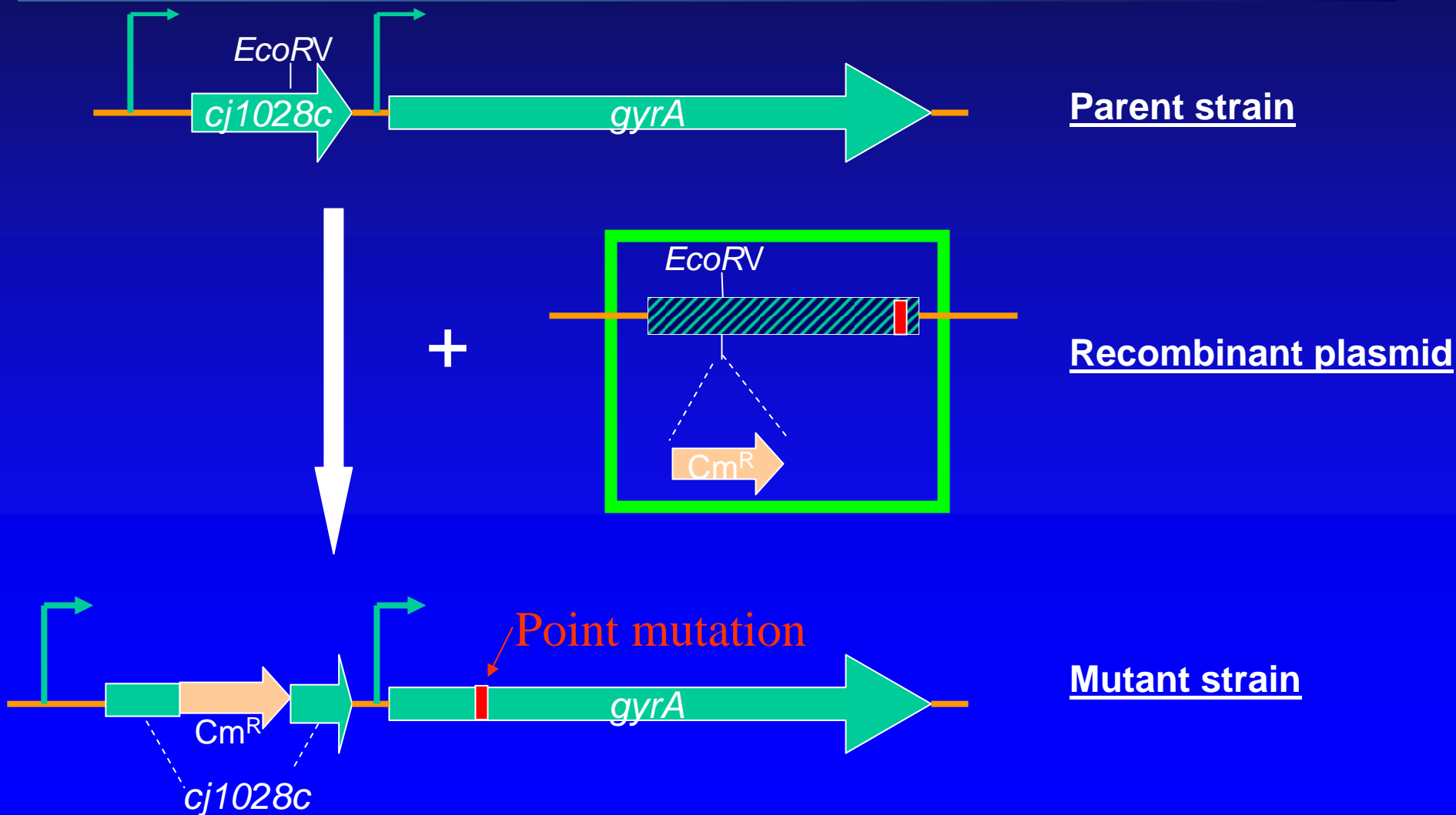


# Role of Target Gene Mutation (*gyrA*) on Antimicrobial Resistance

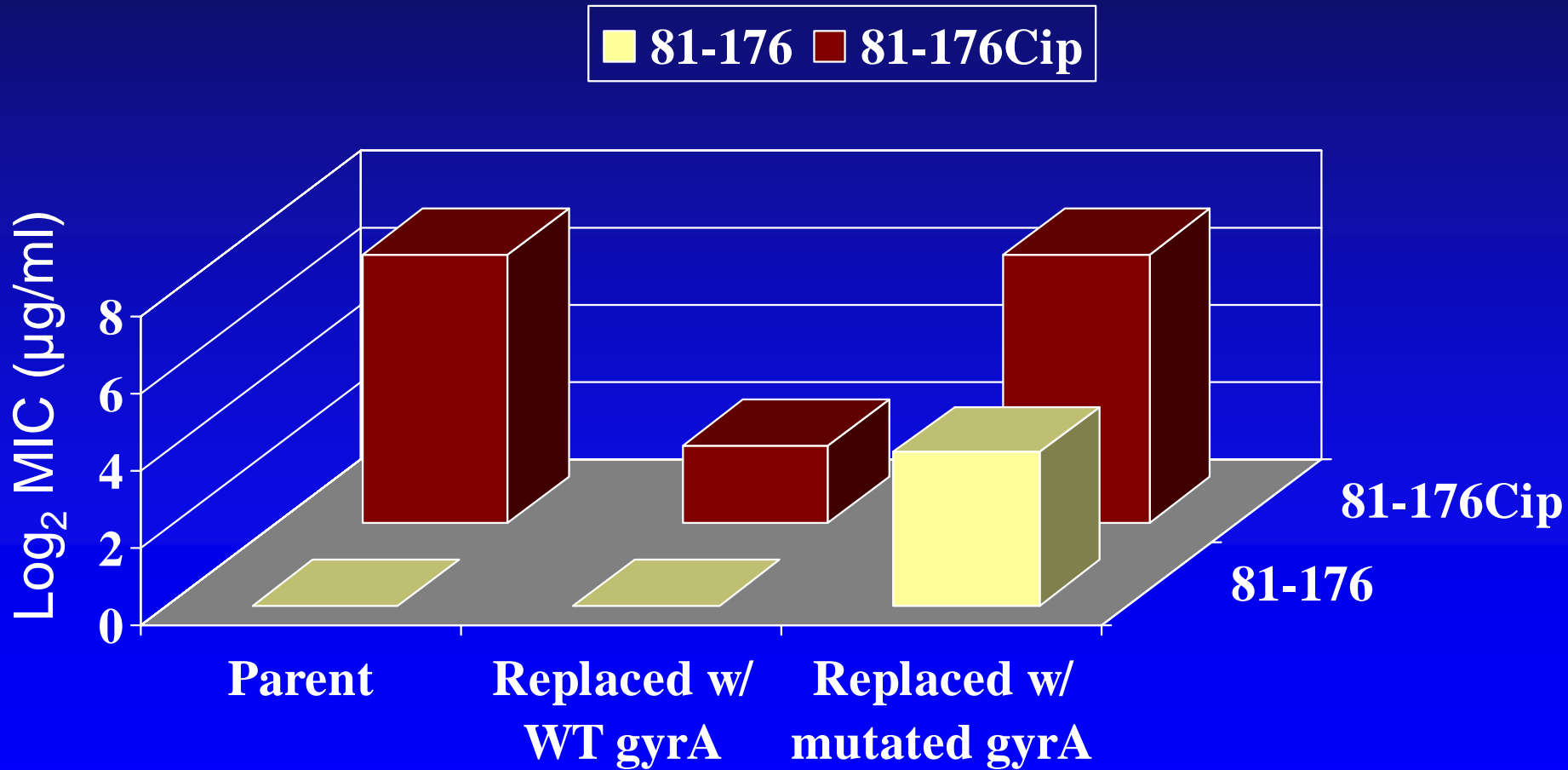
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- Introduce point mutation to *gyrA* in susceptible strains
- Introduce wild type *gyrA* into resistant strain to restore susceptible phenotype of

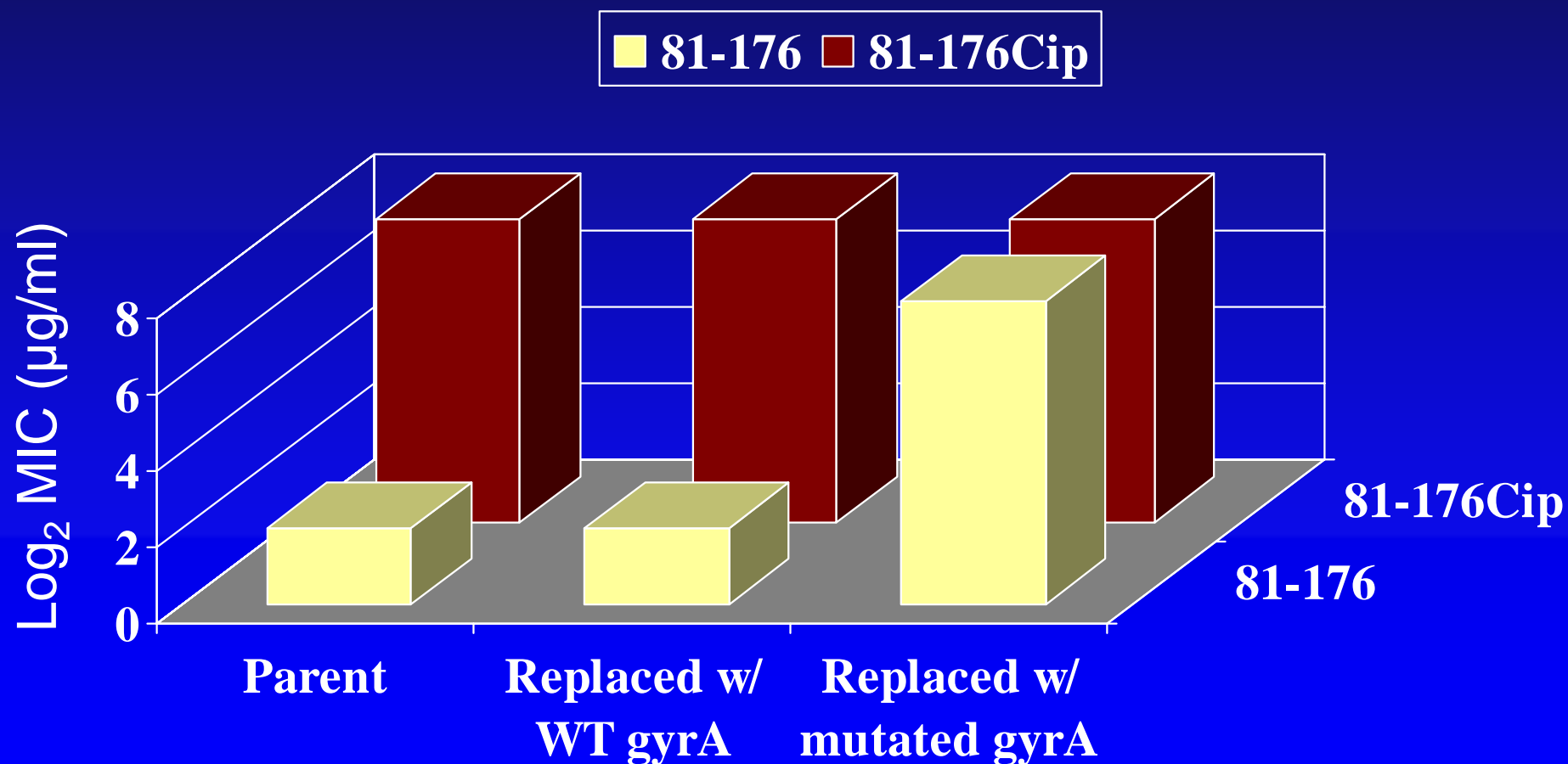
# Construction of *gyrA* mutation



# Ciprofloxacin MICs of 81-176, 81-176Cip and Their Mutants



# Nalidixic Acid MICs of 81-176, 81-176Cip and Their Mutants



# Conclusions

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- *Campylobacter* strains have diverse efflux pump gene profiles
- Although many efflux pumps are present in *Campylobacter*, CmeABC is the only efflux pump among tested important to antibiotic resistance
- Single point mutation (Thr-86-Ile) in *gyrA* of *C. jejuni* directly caused resistance to fluoroquinolones
- These two mechanisms interplay in contributing to antimicrobial resistance of *Campylobacter* species