# *Mechanisms of Aminoglycoside Resistance*

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There are three mechanisms of aminoglycoside resistance:

- reduced uptake or decreased cell permeability
- alterations at the ribosomal binding sites
- production of aminoglycoside modifying enzymes

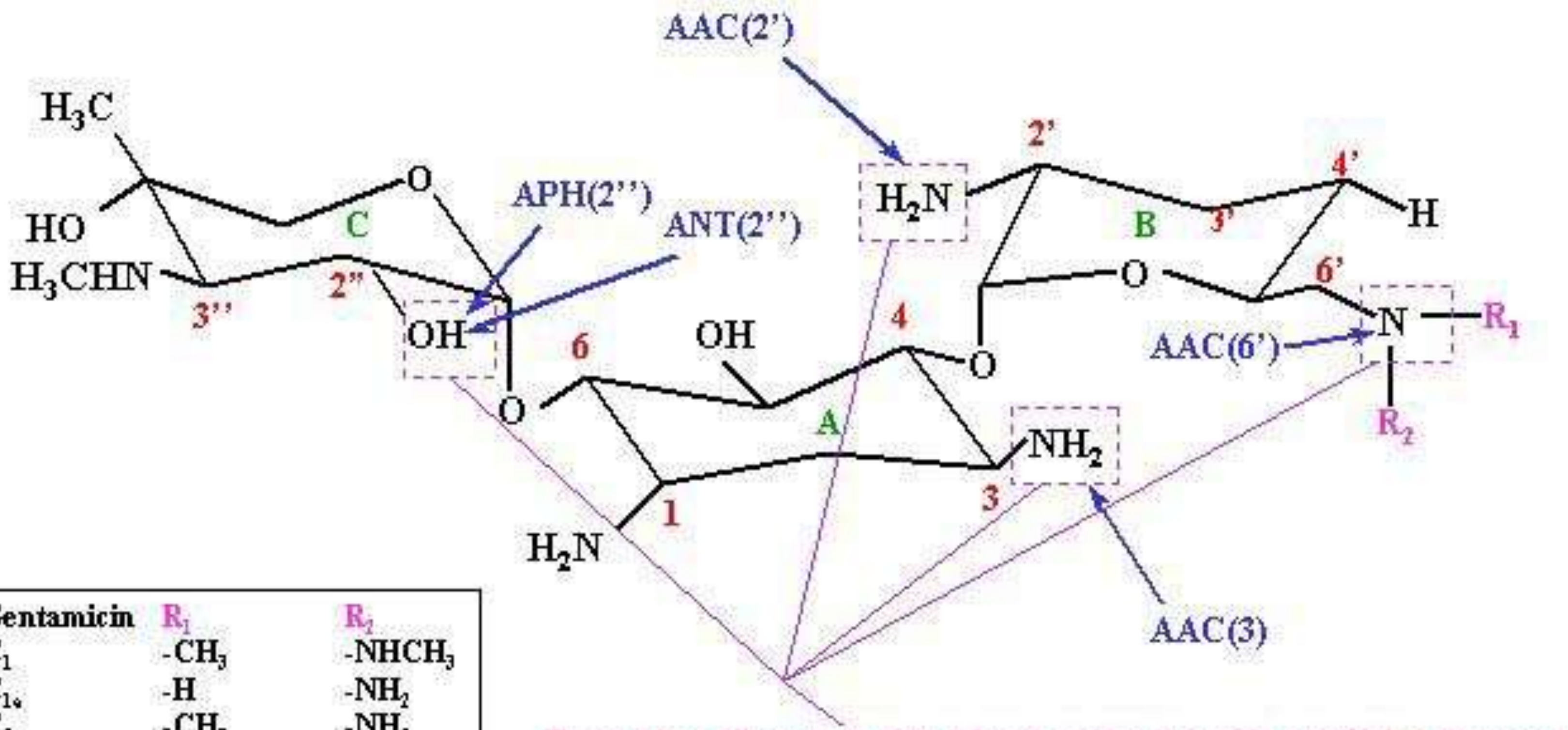
# Aminoglycoside-modifying enzymes

- **N-Acetyltransferases (AAC) –** catalyzes acetyl CoA-dependent acetylation of an amino group
- **O-Adenyltransferases (ANT) –** catalyzes ATP-dependent adenylation of hydroxyl group
- **O-Phosphotransferases (APH) –** catalyzes ATP-dependent phosphorylation of a hydroxyl group

**The nomenclature is defined as follows:**

- AAC, ANT, or APH for the type of enzymatic modification,
- followed by a number in parentheses designating the site of modification.
- The Roman numerals and letters that follow stand for unique resistance profiles and protein designations, respectively.

# Gentamicin

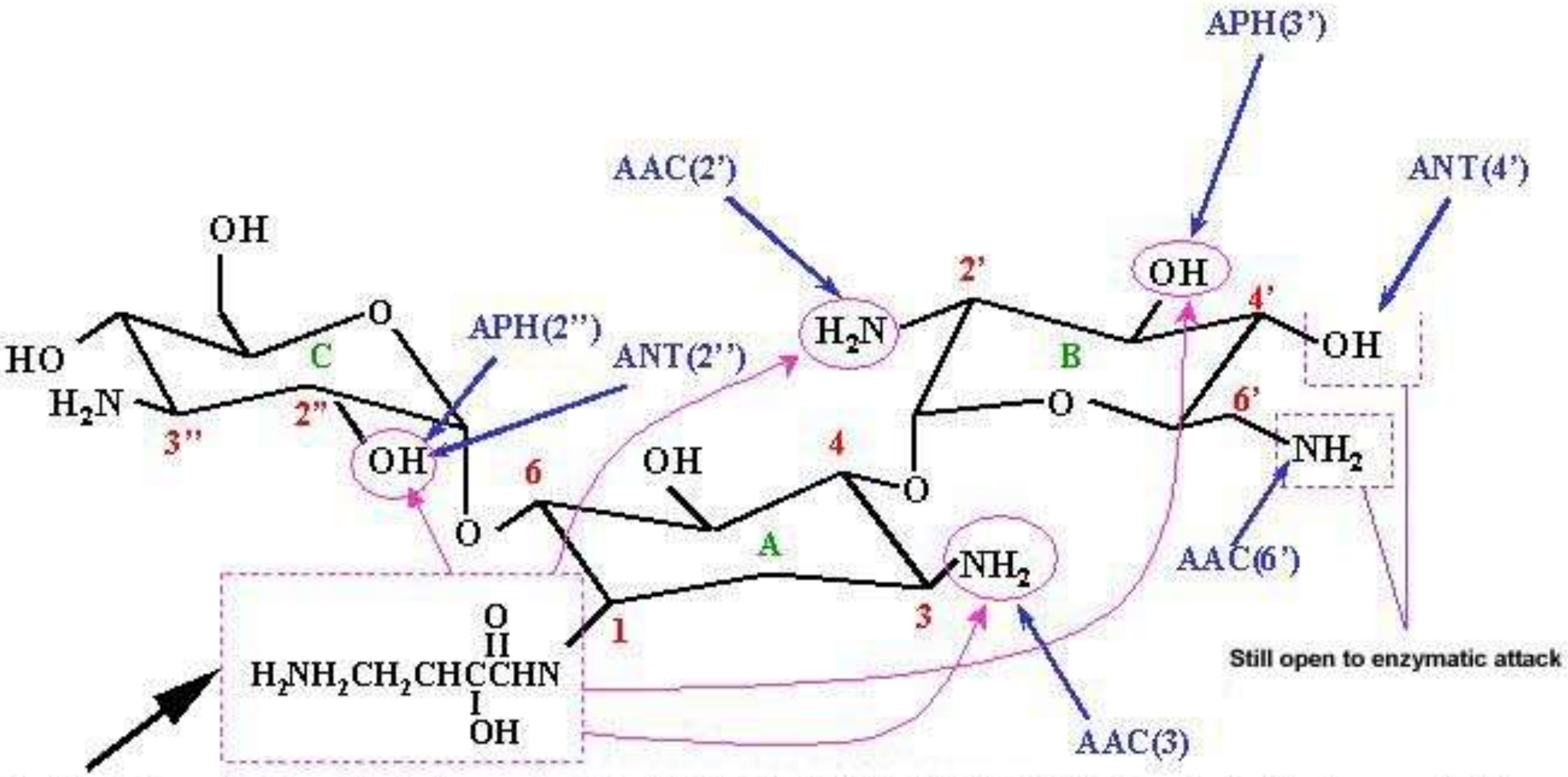


These sites all open to attack from aminoglycoside modifying enzymes

| Gentamicin | $R_1$ | $R_2$ |
|---|---|---|
| $C_1$ | $-CH_3$ | $-NHCH_3$ |
| $C_{1a}$ | -H | $-NH_2$ |
| $C_2$ | $-CH_3$ | $-NH_2$ |
| $C_{2b}$ | -H | $-NHCH_3$ |

Modified from Antimicrob Agents Chemother 1999;43:727-37

# Amikacin



This side chain protects amikacin from attack by AAC(3, 2'), APH (3', 2''), & ANT (2'') - by steric hindrance or folding.

Modified from Antimicrob Agents Chemother 1999;43:727-37

| Enzyme | Genes | Selected Aminoglycoside Substrates | Comments |
|---|---|---|---|
| AAC(3)-I | aac(3)-Ia | Gm | |
| | aac(3)-Ib | | |
| AAC(3)-II | aac(3)-IIa | Gm, Tob | |
| | aac(3)-IIb | | |
| | aac(3)-IIc | | |
| AAC(3)-III | aac(3)-IIIa | Gm, Tob, Km, Neo, Prm | Commonly found in Pseudomonas spp. |
| | aac(3)-IIIb | | Rarely seen in Enterobacteriaceae |
| | aac(3)-IIIc | | |
| AAC(6’)-I | aac(6’)-Ia | Tob, Amk | |
| | aac(6’)-Ib | | |
| | aac(6’)-Ic | | |
| | aac(6’)-Id | | |
| | aac(6’)-Ie | | |
| | aac(6’)-If | | |
| | aac(6’)-Ig | | |
| | aac(6’)-Ih | | |
| | aac(6’)-Ii | | |

| | | | |
|---|---|---|---|
| AAC(6’)-APH(2”) | aac(6’)-aph(2”) | Gm, Tob, Amk | Bifunctional enzyme thought to be restricted to gram positive bacteria. (staphylococci and enterococci) |
| ANT(2”)-I | ant(2”)-Ia | Gm, Tob, Km | Widespread among all gram-negative bacteria |
| | ant(2”)-Ib | | |
| | ant(2”)-Ic | | |
| ANT(3”)-I | ant(3”)-Ia | Sm, Spcm | |
| ANT(4’)-I | ant(4’)-Ia | Tob, Amk | |
| ANT(4’)-II | ant(4’)-IIa | Tob, Amk | |
| ANT(6)-I | ant(6)-Ia | Sm | Found in gram-positive organisms |
| APH(3”)-I | aph(3”)-Ia | Sm | **Cloned from Streptomyces griseus** |
| | aph(3”)-Ib | | |
| APH(6)-I | aph(6)-Ia | Sm | **Cloned from Streptomyces spp.** |
| | aph(6)-Ib | | |
| | aph(6)-Ic | | |
| | | | |

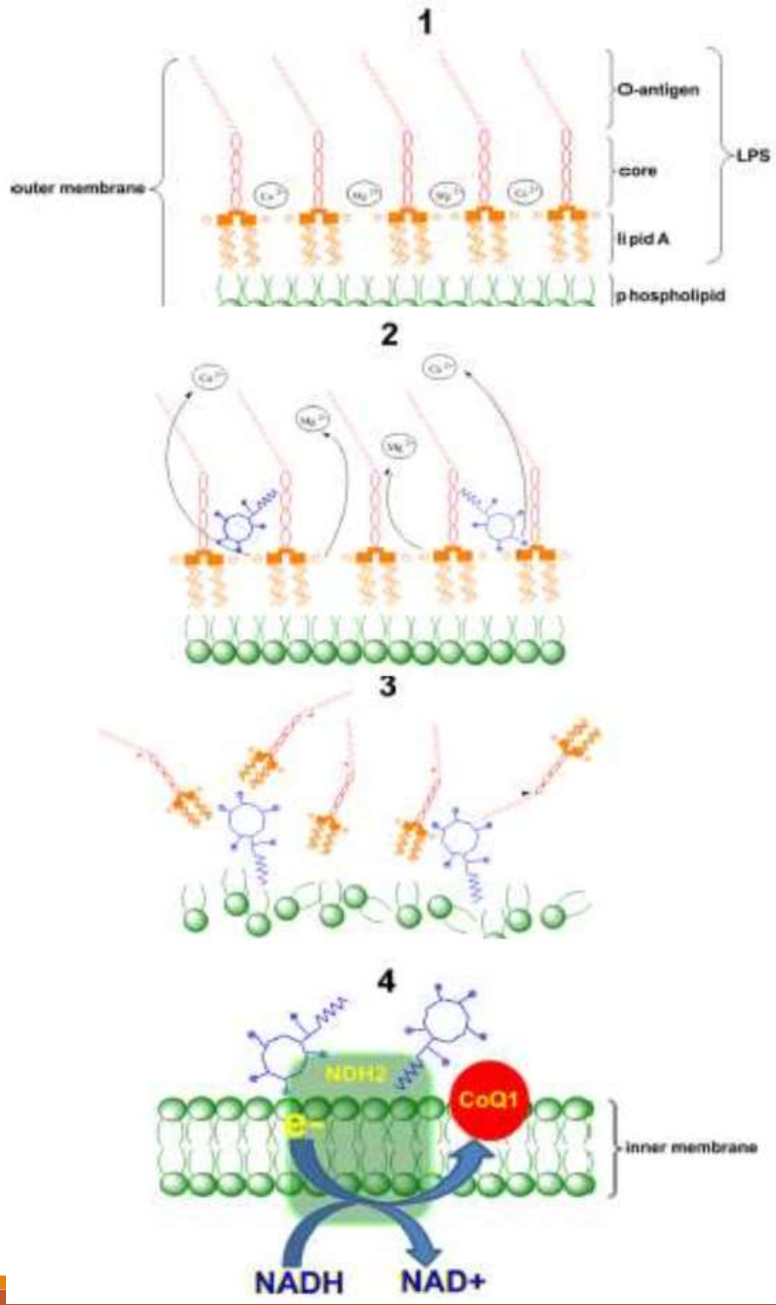
# Examples of aminoglycoside resistance phenotypes of *Enterobacteriaceae*

| Phenotype | Wild | AAC(3)I | AAC(3)II | AAC(3)IV | AAC(6’) | ANT(2’) | APH(3’) |
|---|---|---|---|---|---|---|---|
| Gentamicin | S | R | R | R | S/r | R | S |
| Netilmicin | S | S | R | R | R | S | S |
| Tobramycin | S | S | R | R | R | R | S |
| Amikacin | S | S | S | S | R | S | S |
| Kanamycin | S | S | R | r | R | R | R |
| Neomycn | S | S | S | R | R | S | R |

# Plasmid-Mediated 16S rRNA Methylases Responsible for aminoglycoside Resistance

- It confers very-high level resistance to all aminoglycosides that are currently available for parenteral formulation.
- Six distinct genes, *rmtA*, *rmtB*, *rmtC*, *rmtD*, *armA*, and *npmA*
- **NpmA** is the only enzyme among them that methylates residue **A1408**, whereas the others methylate residue **G1405**, both within the aminoacyl site (A site) of the 16S rRNA

# Colistin resistance



1. Fixation

2. Displacement of divalent cation ($Ca^{2+}$ et $Mg^{2+}$)

3. Destabilisation of the outer membrane

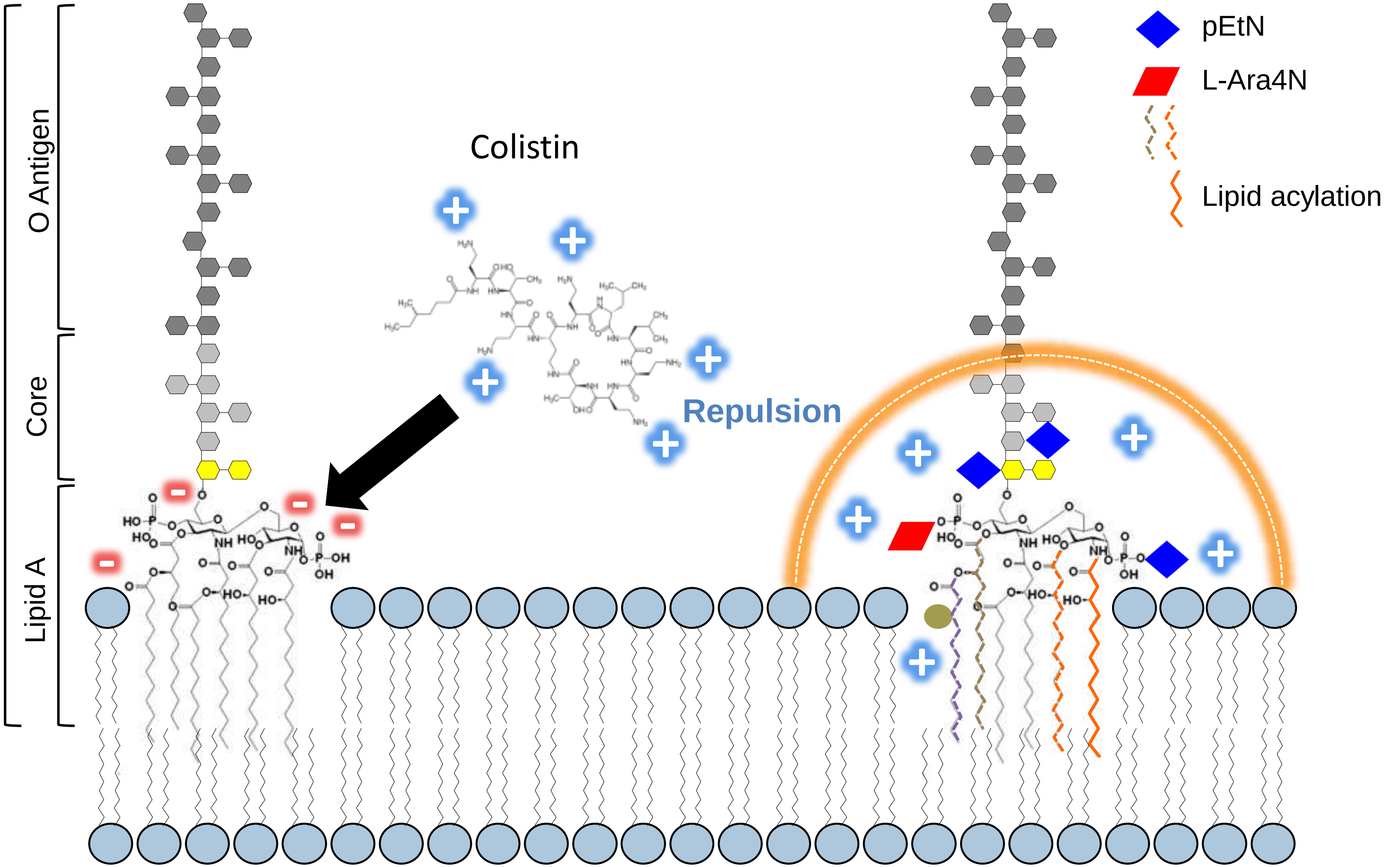
4. Penetration throughout the inner membrane and inhibition of the respiratory enzymes NDH2

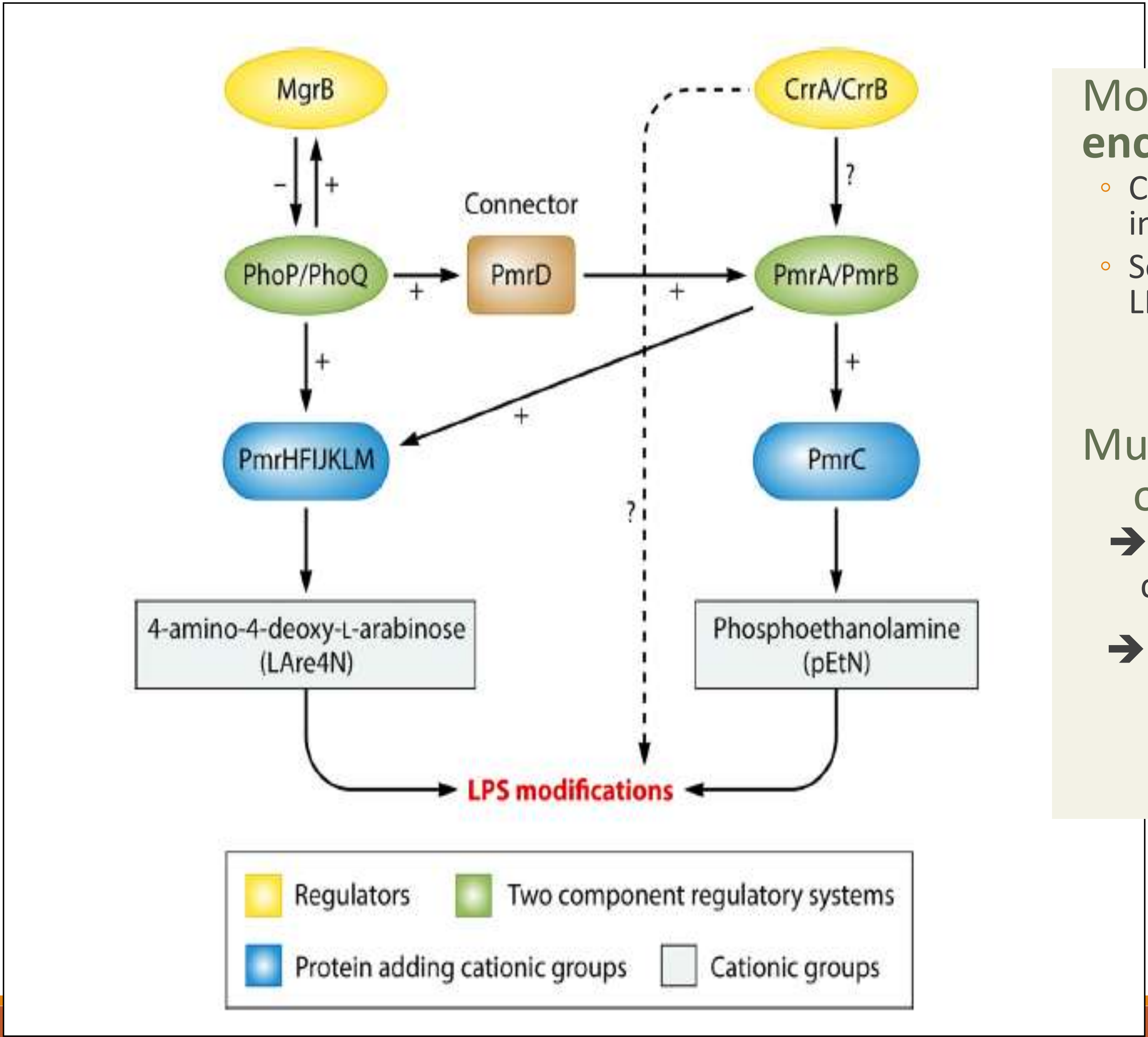
# LPS modifications : the main mechanism of resistance to colistin

- Addition of 4-amino-4-deoxy-L-arabinose (LAra4N) and or phosphoethanolamine (pEtN) to lipid A ➔ Increase of positive charges ➔ decreased affinity for LPS

- Synthesis of L-Ara4N and pEtN mediated by PmrA / PmrB, PhoP / PhoQ, and *mgrB* gene

# Mechanisms of resistance to colistin





## Modification of LPS by **chromosomal encoded** resistance mechanisms

- Complex regulation pathways of LPS modification in Gram-negative
- Several genes/operons involved in modification of LPS

  (addition of cationic groups)

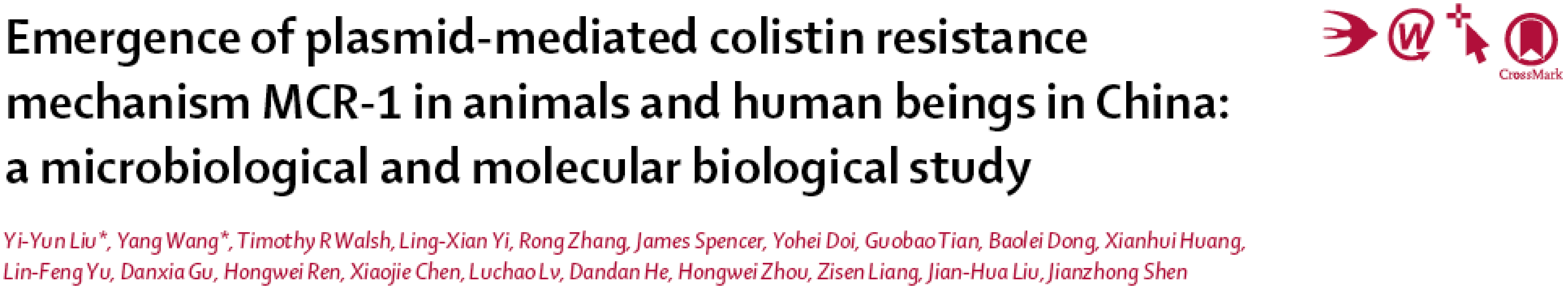
## Mutations in two-component systems or deletions/insertions in regulators

➔Increased expression of proteins adding cationic groups

➔ Diminution of negative charge of LPS leads to decrease affinity of colistin to LPS and to resistance

Addition of 4-Amino-4deoxy-L-ARA leads to higher resistance rate than addition of pEtn

# Since november 2015, acquired resistance to colistin involved **ALSO plasmid** mediated resistance



## Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study

Yi-Yun Liu*, Yang Wang*, Timothy R Walsh, Ling-Xian Yi, Rong Zhang, James Spencer, Yohei Doi, Guobao Tian, Baolei Dong, Xianhui Huang, Lin-Feng Yu, Danxia Gu, Hongwei Ren, Xiaojie Chen, Luchao Lv, Dandan He, Hongwei Zhou, Zisen Liang, Jian-Hua Liu, Jianzhong Shen

**MCR-1**

Plasmid encoded phosphoethanolamine transferase