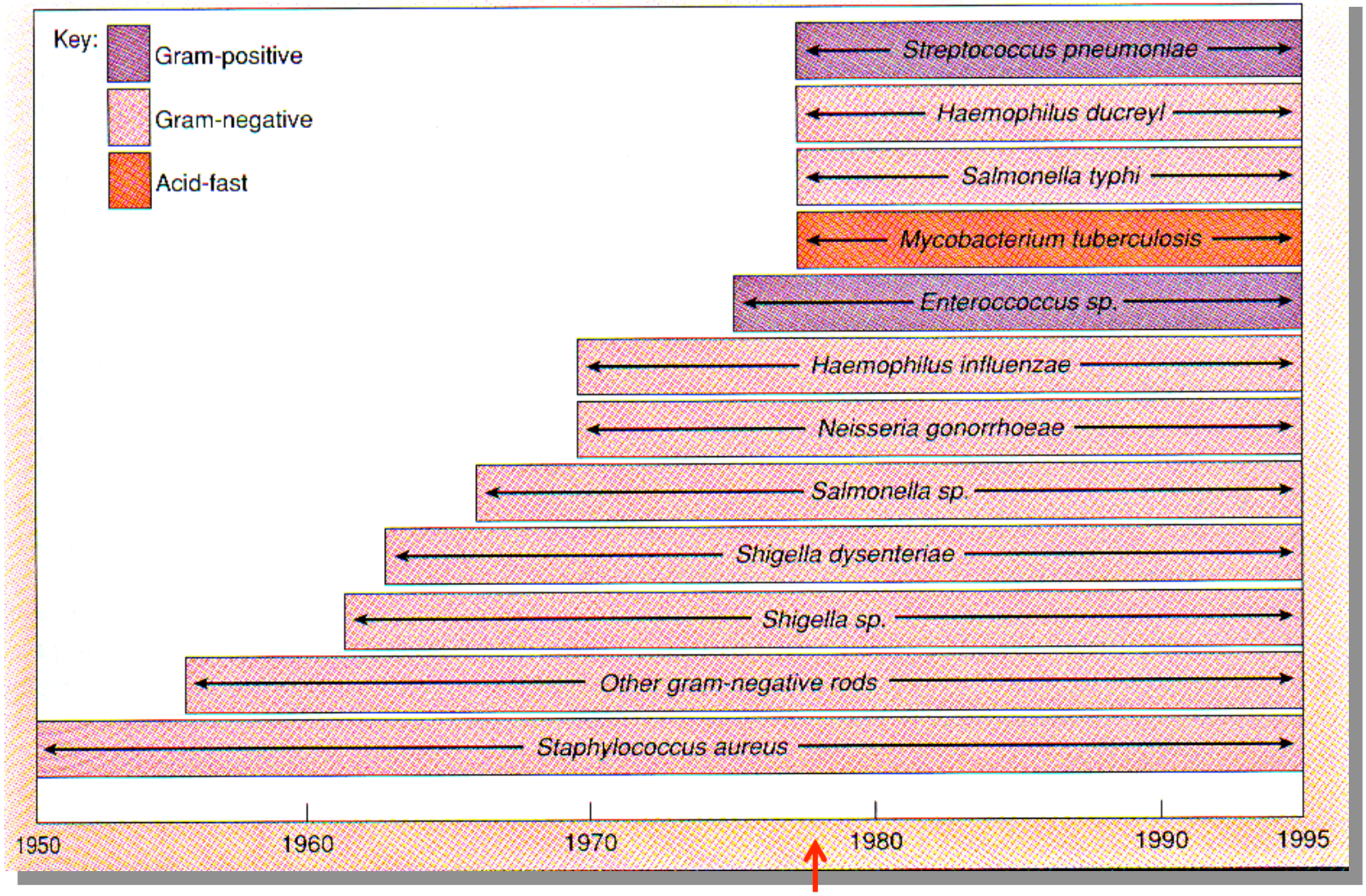
Part 2.

Resistance to Antibiotics

New developments

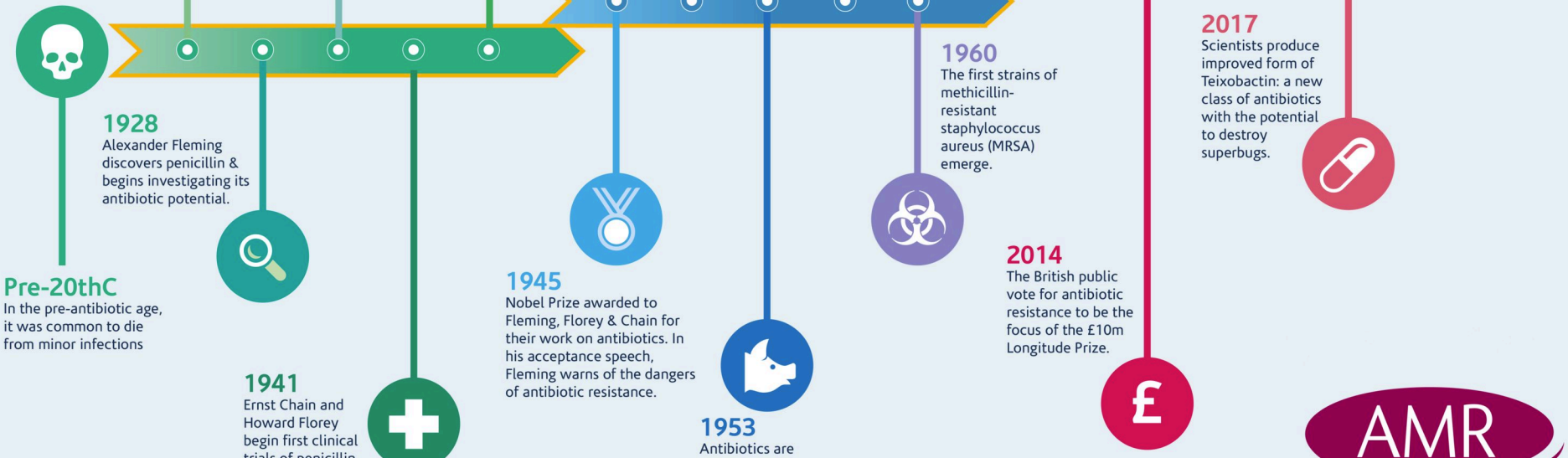

What Causes Antibiotic Resistance?

Cronologia da Resistência a Antimicrobianos



Staphylococcus aureus meticilina-resistente (MRSA)

A BRIEF HISTORY OF ANTIBIOTICS & RESISTANCE



Pre-20thC
In the pre-antibiotic age, it was common to die from minor infections

1910
The first antimicrobial drug, Salvarsan, is synthesised from clothes dye & used to treat syphilis.

1928
Alexander Fleming discovers penicillin & begins investigating its antibiotic potential.

1935
Sulfanamide drugs are released, becoming the first antibiotics to be used systematically.

1941
Ernst Chain and Howard Florey begin first clinical trials of penicillin.

1943
Allied WW2 soldiers begin receiving supplies of antibiotics, saving thousands of lives.

1945
Nobel Prize awarded to Fleming, Florey & Chain for their work on antibiotics. In his acceptance speech, Fleming warns of the dangers of antibiotic resistance.

1948
Penicillin-resistant staphylococcus becomes a global pandemic.

1953
Antibiotics are introduced to animal feed in Europe.

1959
Methicillin antibiotics are invented to combat penicillin-resistant staphylococcus.

1960
The first strains of methicillin-resistant staphylococcus aureus (MRSA) emerge.

1987
Daptomycin is invented. After this, no new classes of antibiotics are discovered for ~30 years.

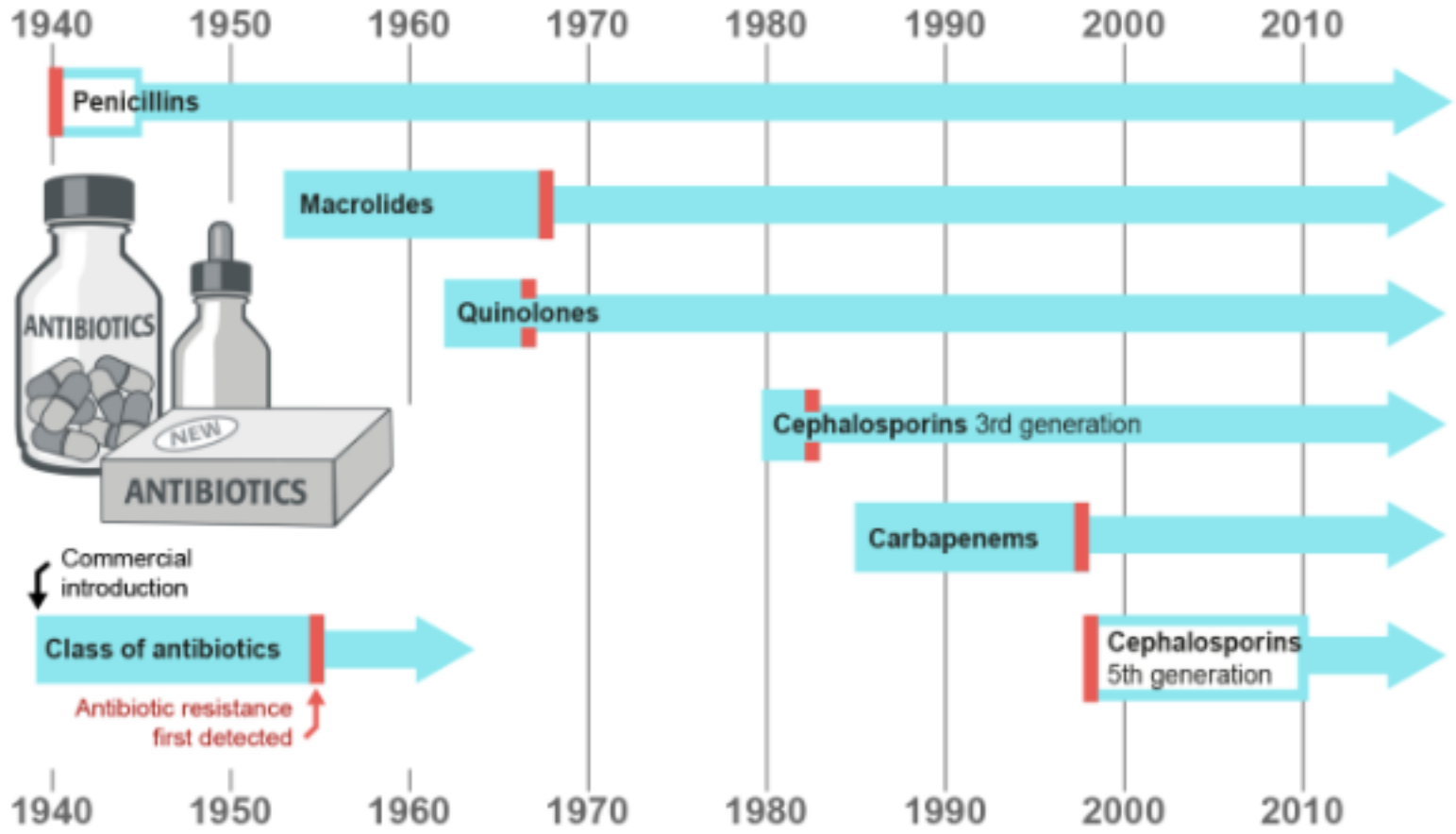
2014
The British public vote for antibiotic resistance to be the focus of the £10m Longitude Prize.

2015
The World Health Organisation (WHO) launches the first Antibiotics Awareness Week.

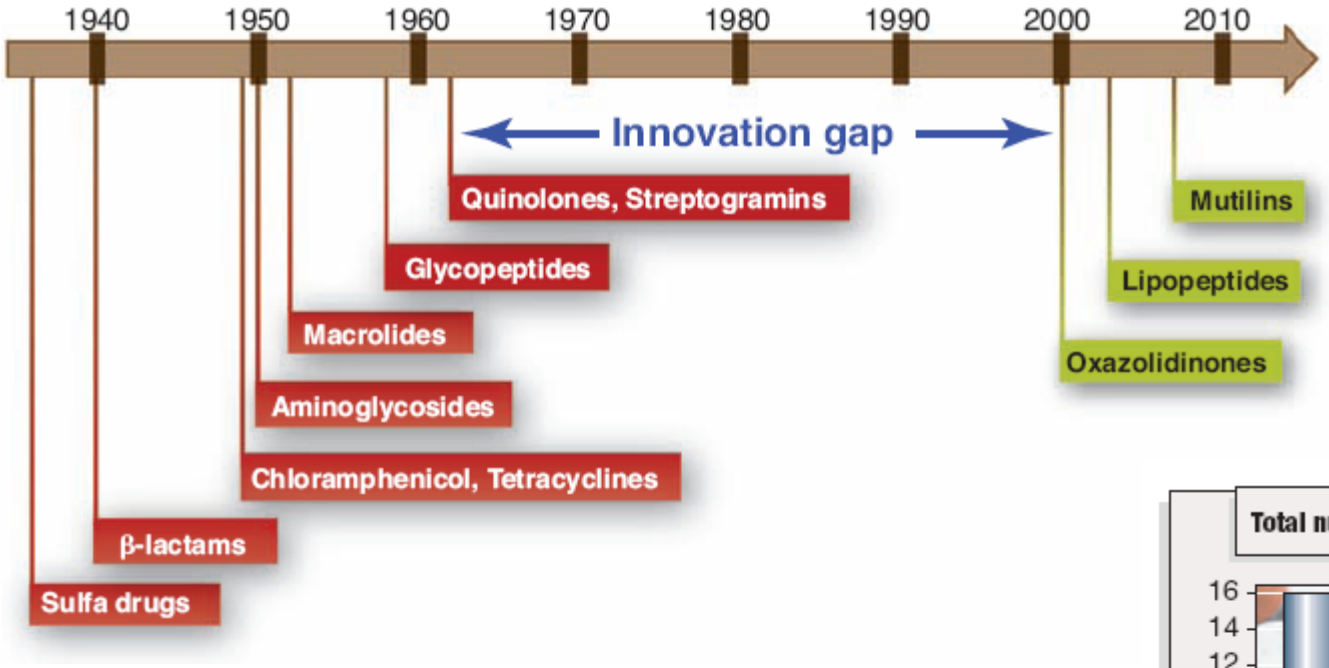
2017
Scientists produce improved form of Teixobactin: a new class of antibiotics with the potential to destroy superbugs.

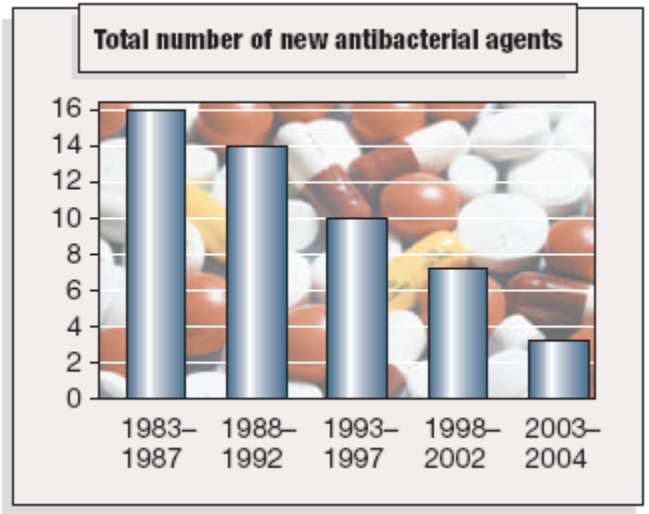
Present Day
Individuals, researchers & policymakers are working together to fight antibiotic resistance.

AMR
Tackling Antimicrobial Resistance
a cross council initiative


1940
1950
1960
1970
1980
1990
2000
2010
Penicillins
Macrolides
Quinolones
Cephalosporins 3rd generation
Carbapenems
Cephalosporins
5th generation
ANTIBIOTICS
NEW
ANTIBIOTICS
Commercial
introduction
Class of antibiotics
Antibiotic resistance
first detected

Desenvolvimento de Novas Drogas



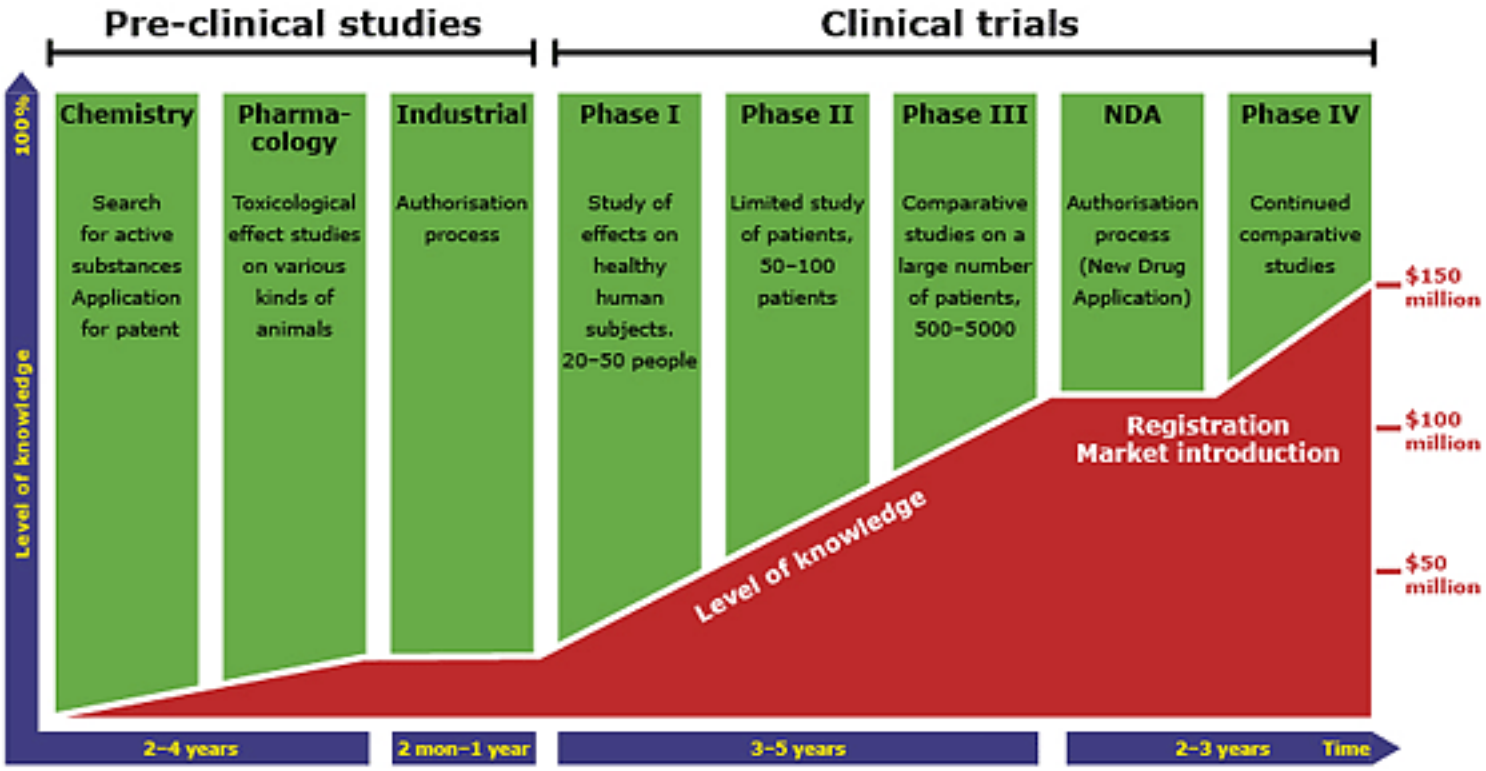


Uso Indiscriminado de Antibióticos em Animais

"Estudos apontam relação entre uso de antibióticos em animais e resistência a medicamentos em seres humanos"

Exemplo: Animais de corte tratados com o glicopeptídeo avoparcina constituem um potencial reservatório de infecção por *Enterococcus* resistente à vancomicina (VRE) em humanos.

Desenvolvimento de Novas Drogas



CAUSES OF ANTIBIOTIC RESISTANCE

HANDLE ANTIBIOTICS WITH CARE

Antibiotic resistance happens when bacteria change and become resistant to the antibiotics used to treat the infections they cause.

Over-prescribing of antibiotics

Patients not finishing their treatment

Over-use of antibiotics in livestock and fish farming

Poor infection control in hospitals and clinics

Lack of hygiene and poor sanitation

Lack of new antibiotics being developed

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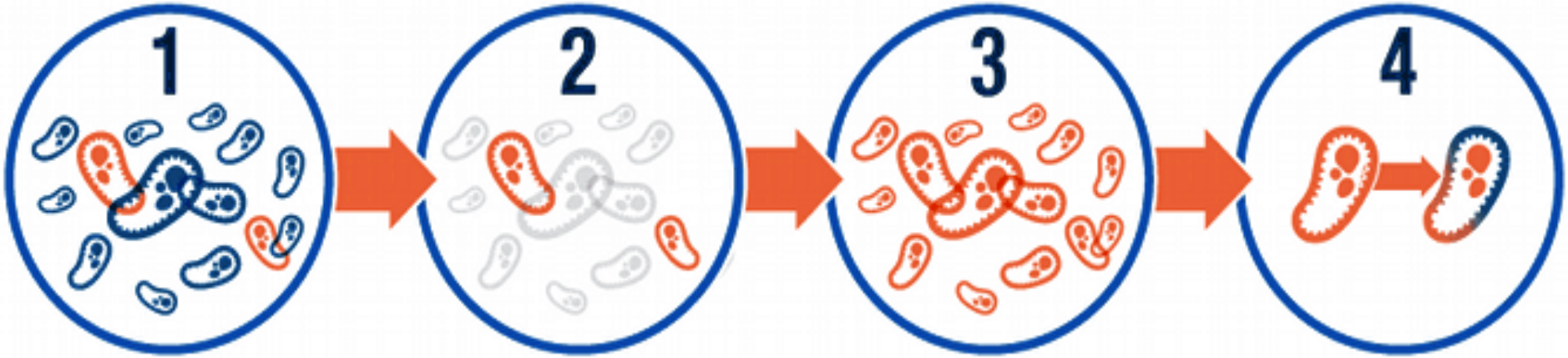
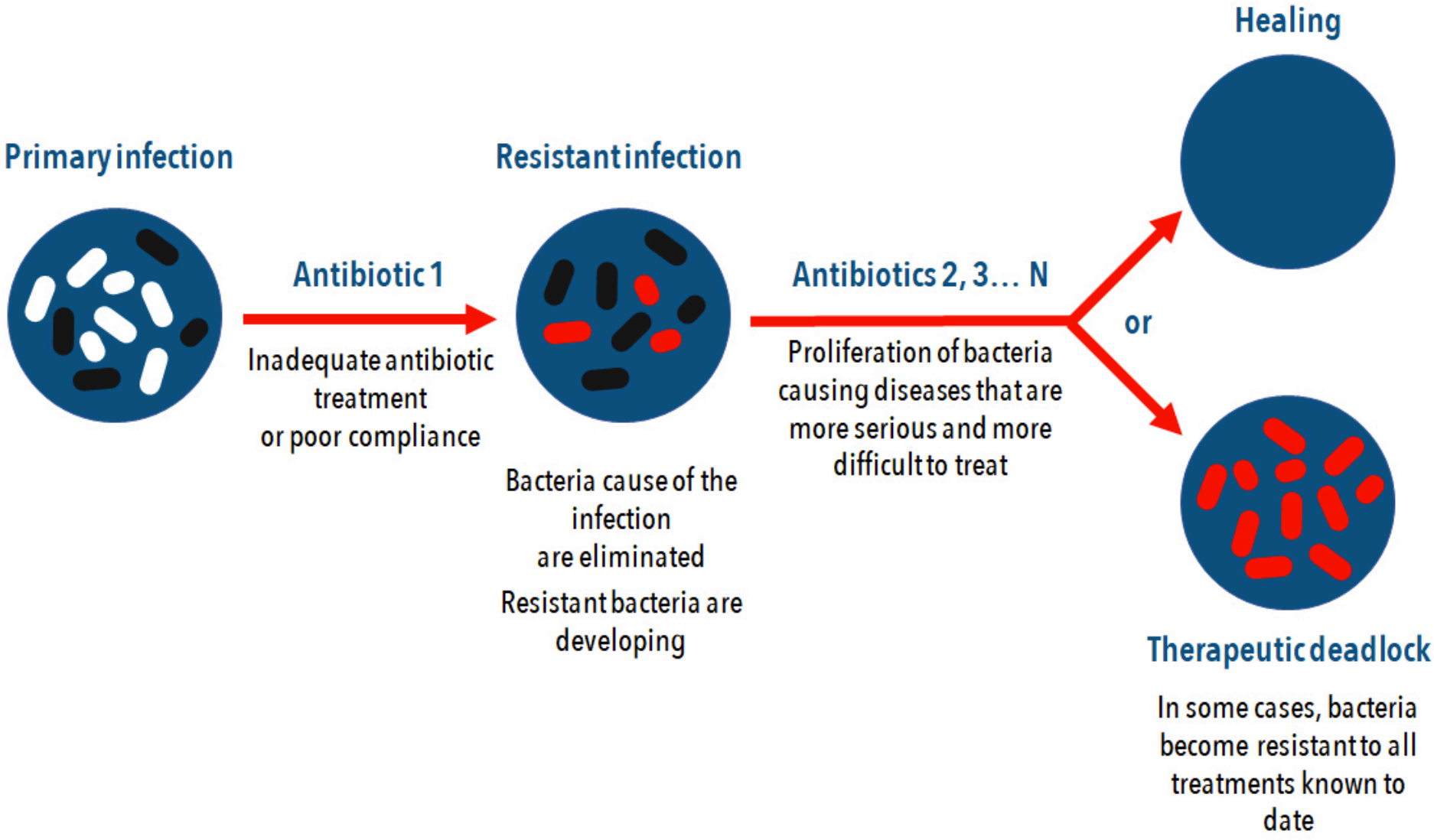
www.who.int/drugresistance

#AntibioticResistance

World Health Organization

How does antibiotic resistance occur?




Primary infection
Antibiotic 1
Inadequate antibiotic treatment or poor compliance
Resistant infection
Bacteria cause of the infection are eliminated
Resistant bacteria are developing
Antibiotics 2, 3… N
Proliferation of bacteria causing diseases that are more serious and more difficult to treat
or
Healing
Therapeutic deadlock
In some cases, bacteria become resistant to all treatments known to date

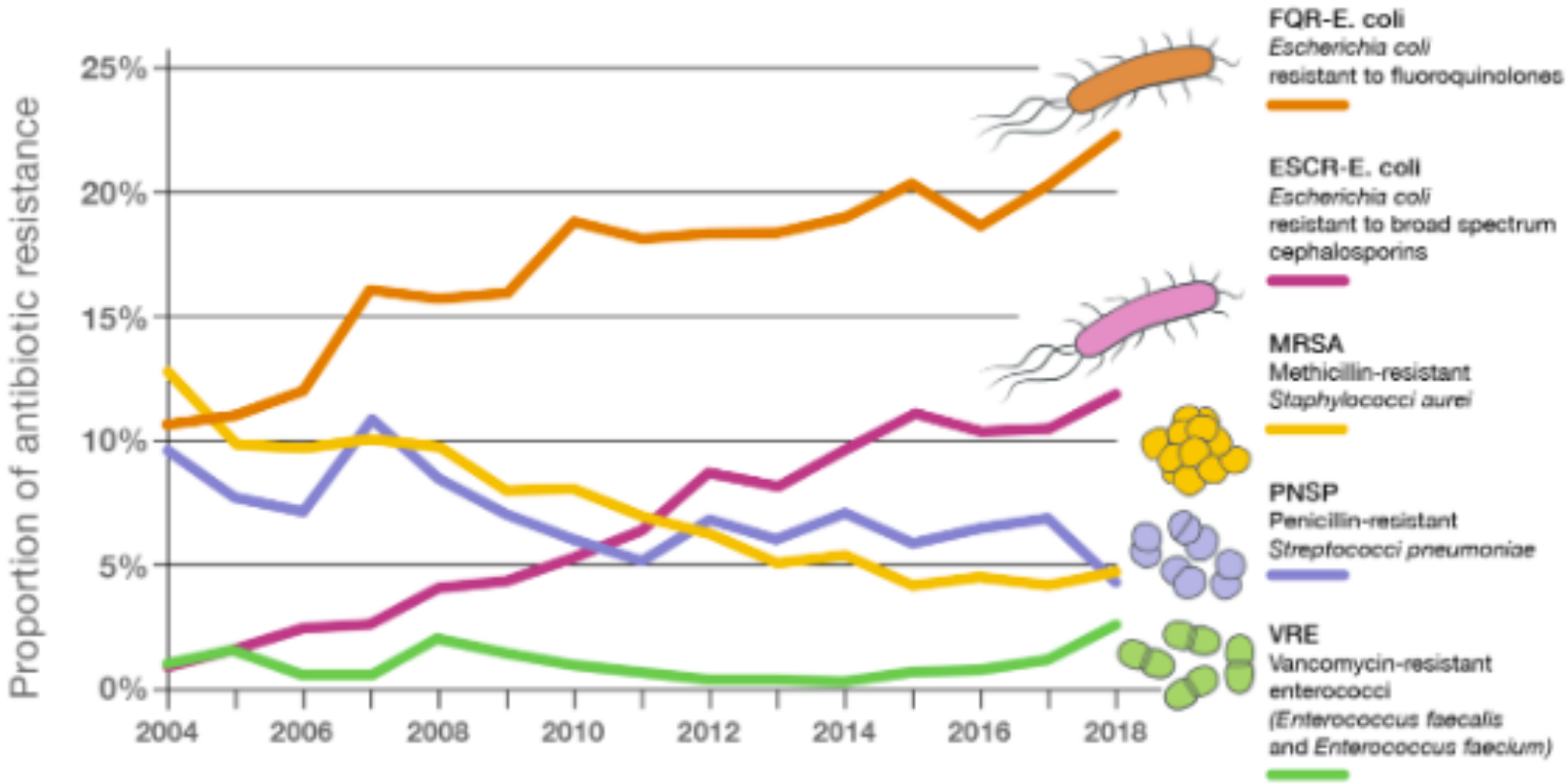
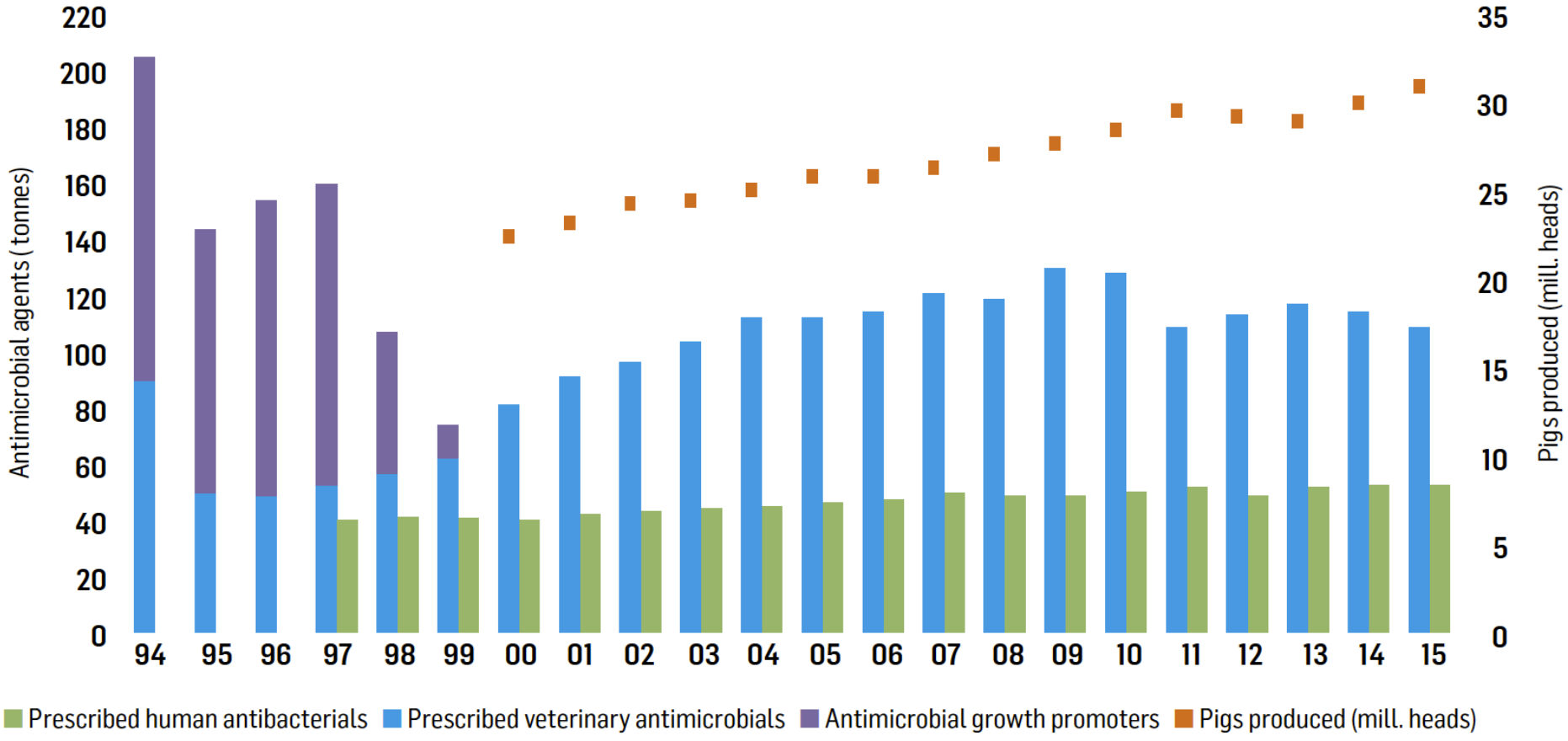

Proportion of antibiotic resistance
25%
20%
15%
10%
5%
0%
2004
2006
2008
2010
2012
2014
2016
2018
FQR-E. coli
Escherichia coli
resistant to fluoroquinolones
ESCR-E. coli
Escherichia coli
resistant to broad spectrum
cephalosporins
MRSA
Methicillin-resistant
Staphylococci aurei
PNSP
Penicillin-resistant
Streptococci pneumoniae
VRE
Vancomycin-resistant
enterococci
(Enterococcus faecalis
and Enterococcus faecium)

Figure 1

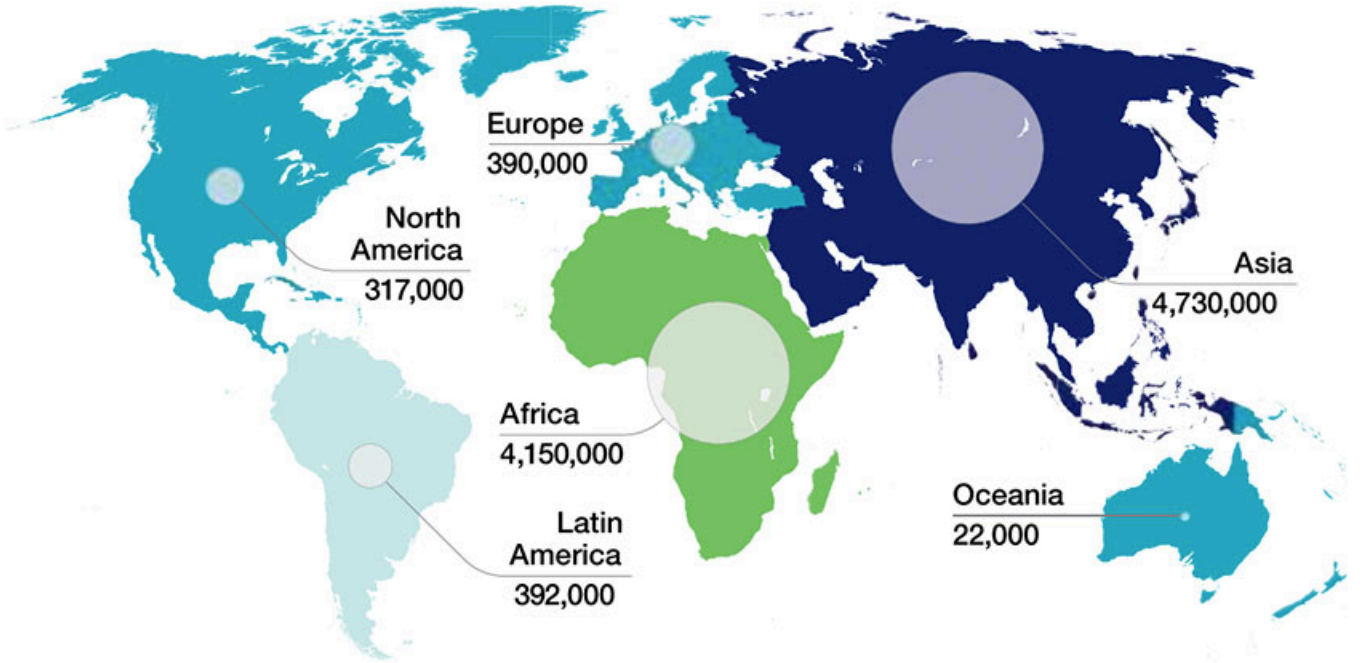
Prescribed antimicrobial agents for human and all animal species

Danmap 2015



Source: Danish Integrated Antimicrobial Resistance Monitoring and Research Programme, 2015

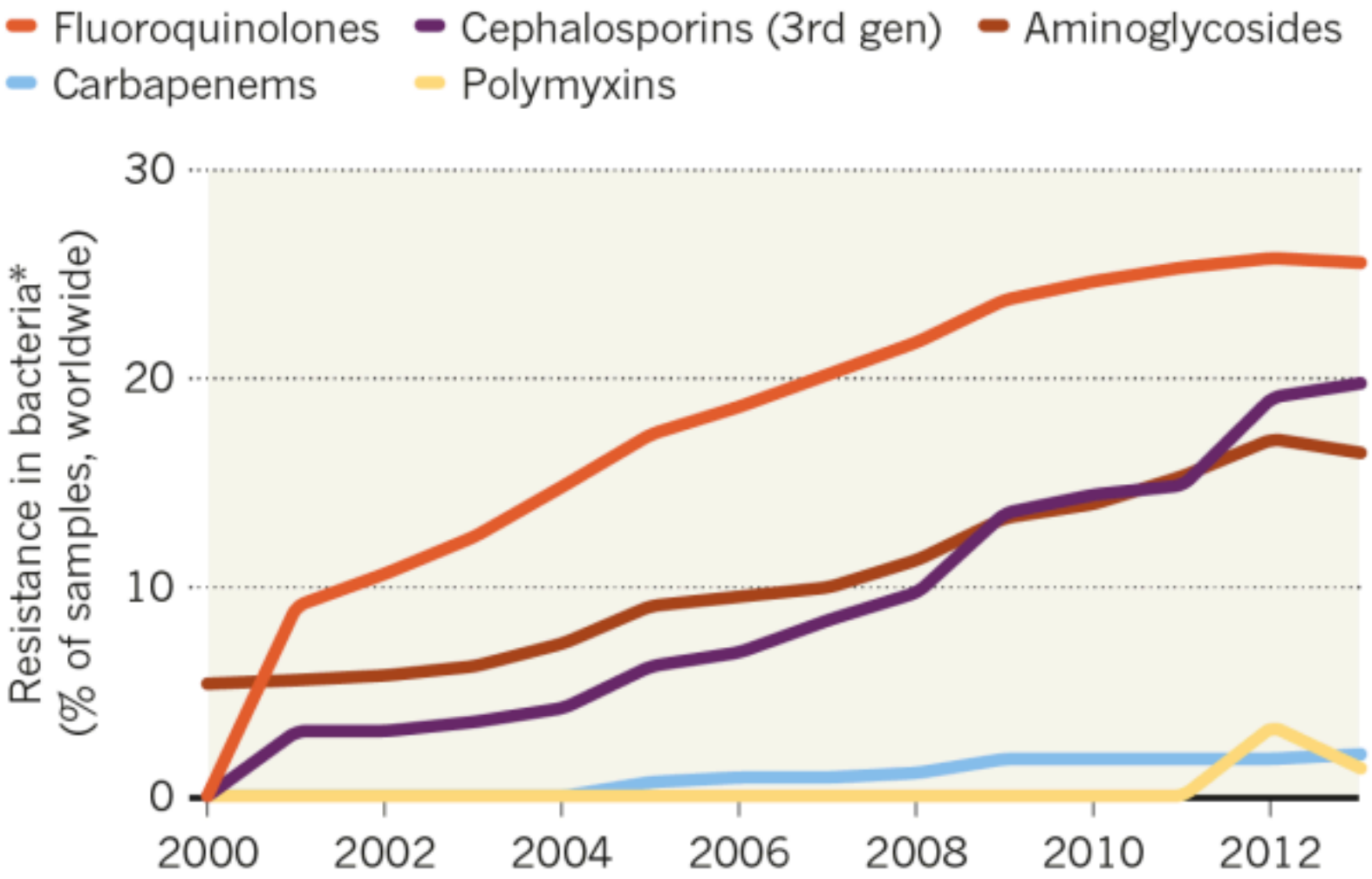
Deaths attributable to AMR every year by 2050



Source: Review on Antimicrobial Resistance

THE SPREAD OF ANTIBIOTIC RESISTANCE

An increasing proportion of bacteria display resistance to common antibiotics.

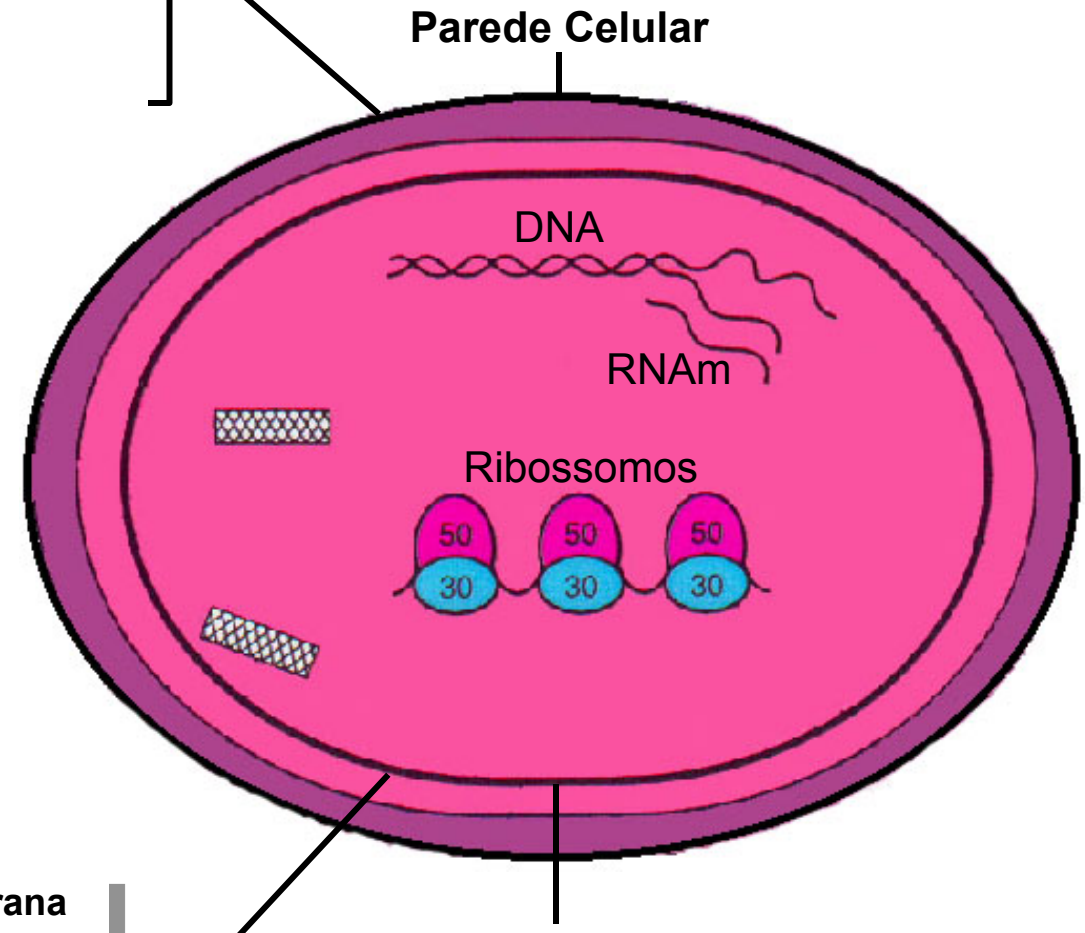


*Enterobacteriae, including *Escherichia coli*, *Klebsellia pneumonia*, *Enterobacter* and *Salmonella*

Inibidores da Síntese de Parede Celular e Organização de Membrana

Síntese da Parede Celular

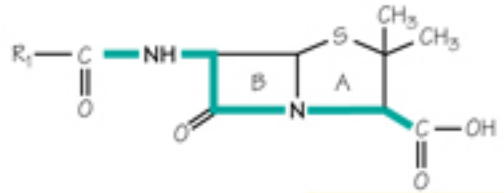
- ★ Cicloserina
- ★ Vancomicina
- ★ Bacitracina
- ★ Penicilinas - (β-lactâmicos)
- ★ Cefalosporinas
- ★ Monobactâmicos
- ★ Carbapenêmicos

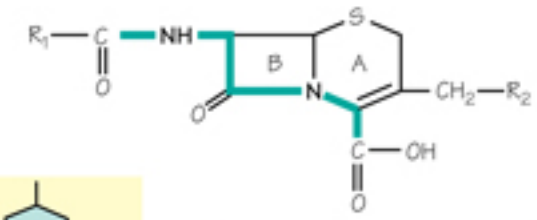


Estrutura da Membrana Citoplasmática

- ★ Polimixinas

β-lactams





Penicillins

PENICILLINASE RESISTANT

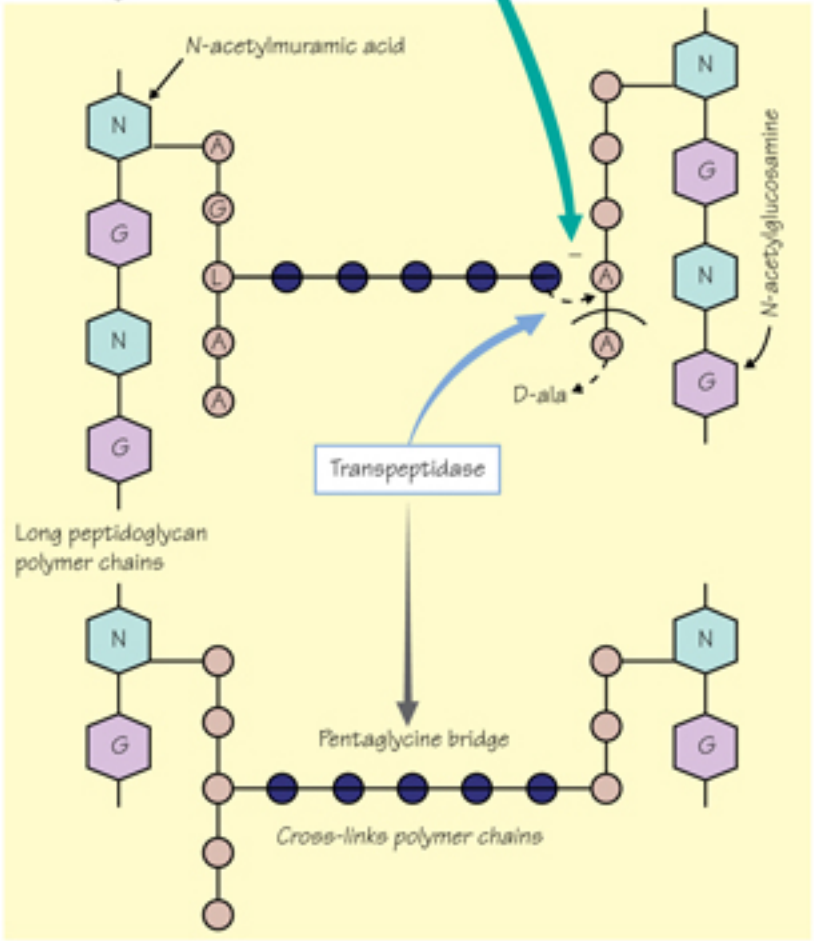
BROAD SPECTRUM

ANTIPSEUDOMONAL

Other β-lactams

CARBAPENEMS

MONOBACTAMS

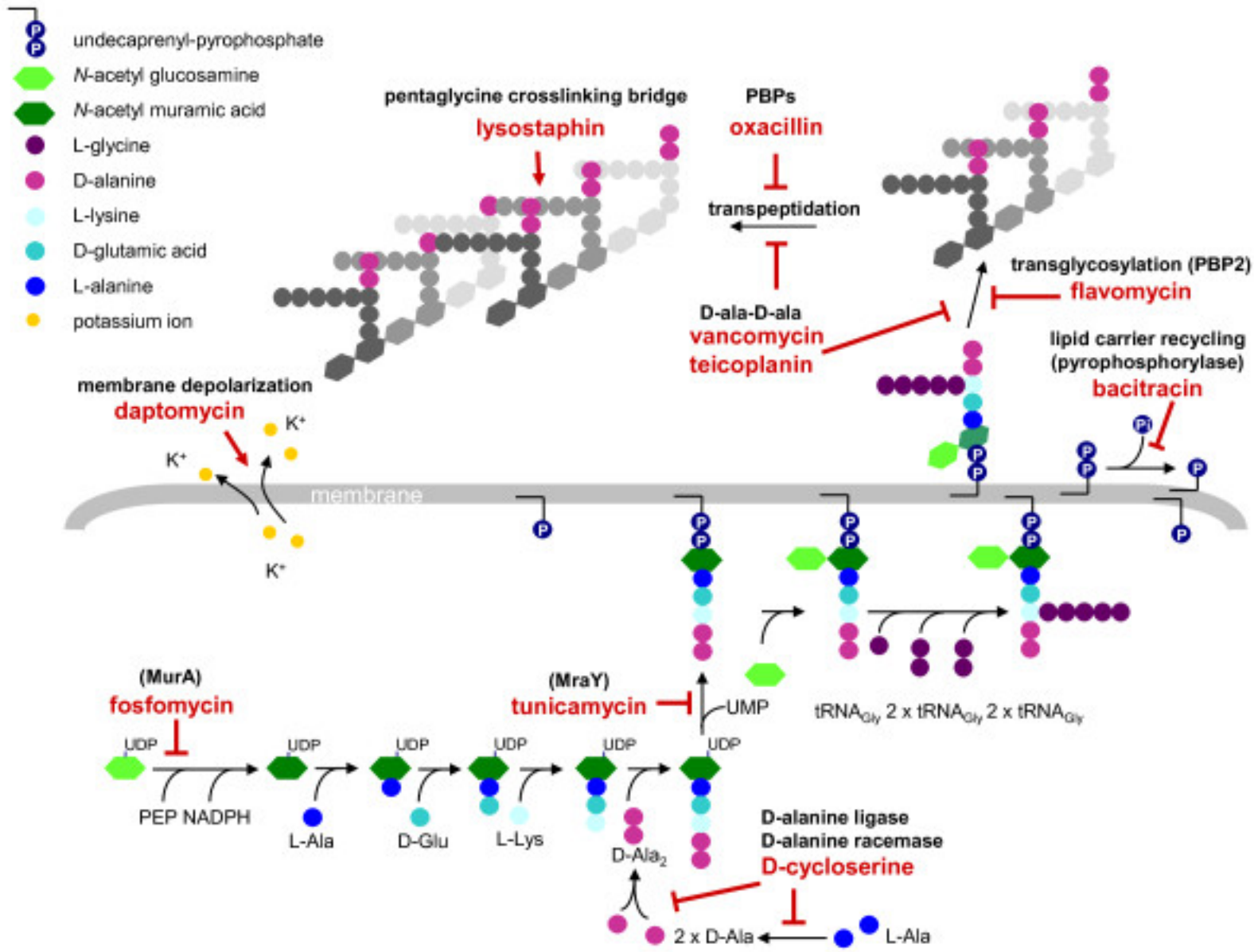


Cephalosporins

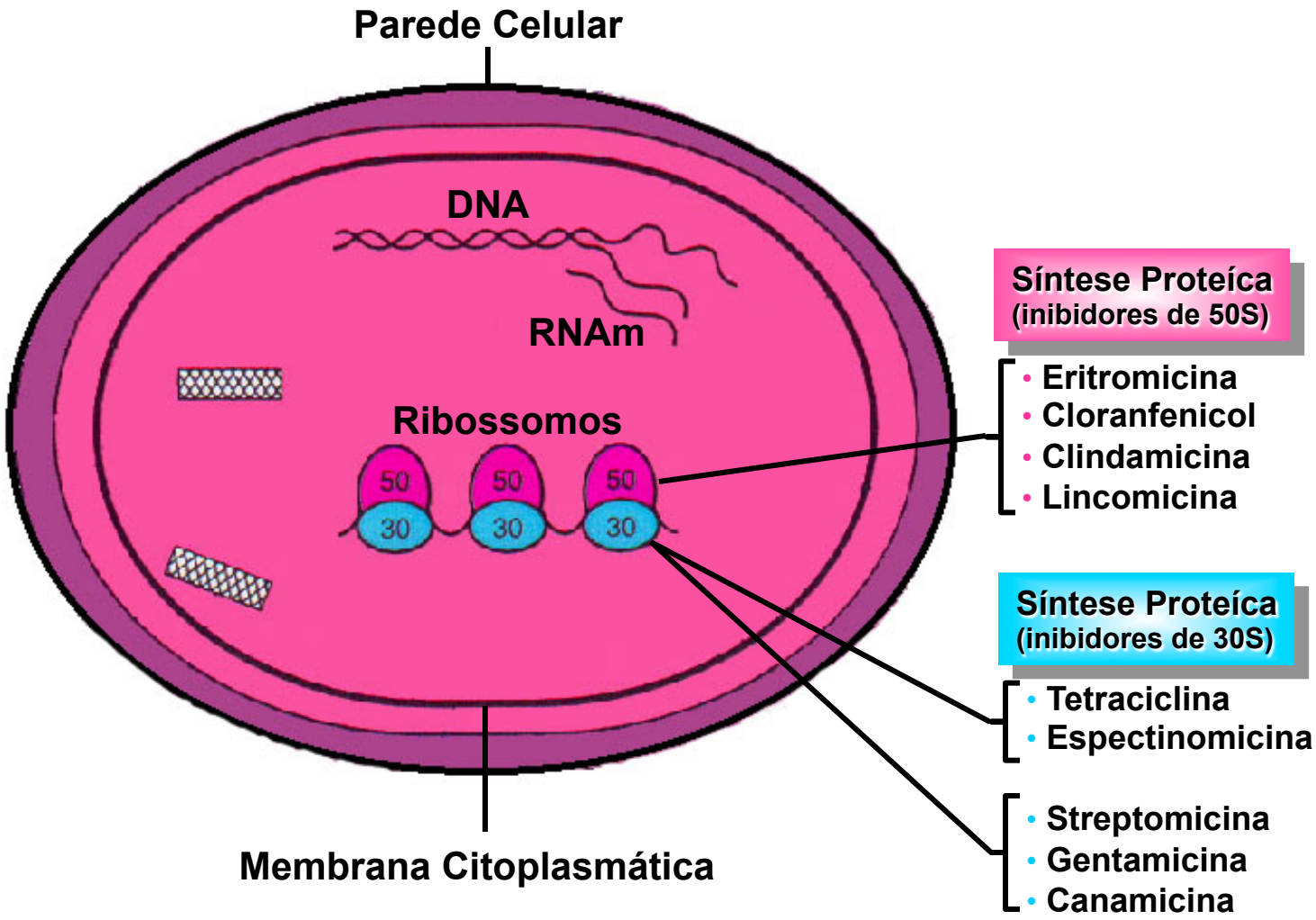
ORALLY ACTIVE

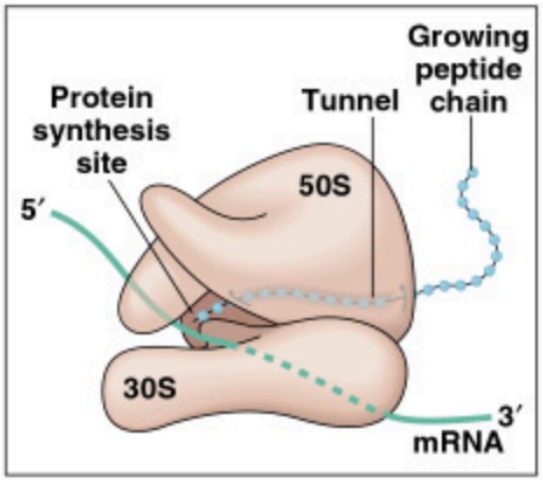
EARLY PARENTERAL AGENTS

NEWER EXTENDED SPECTRUM

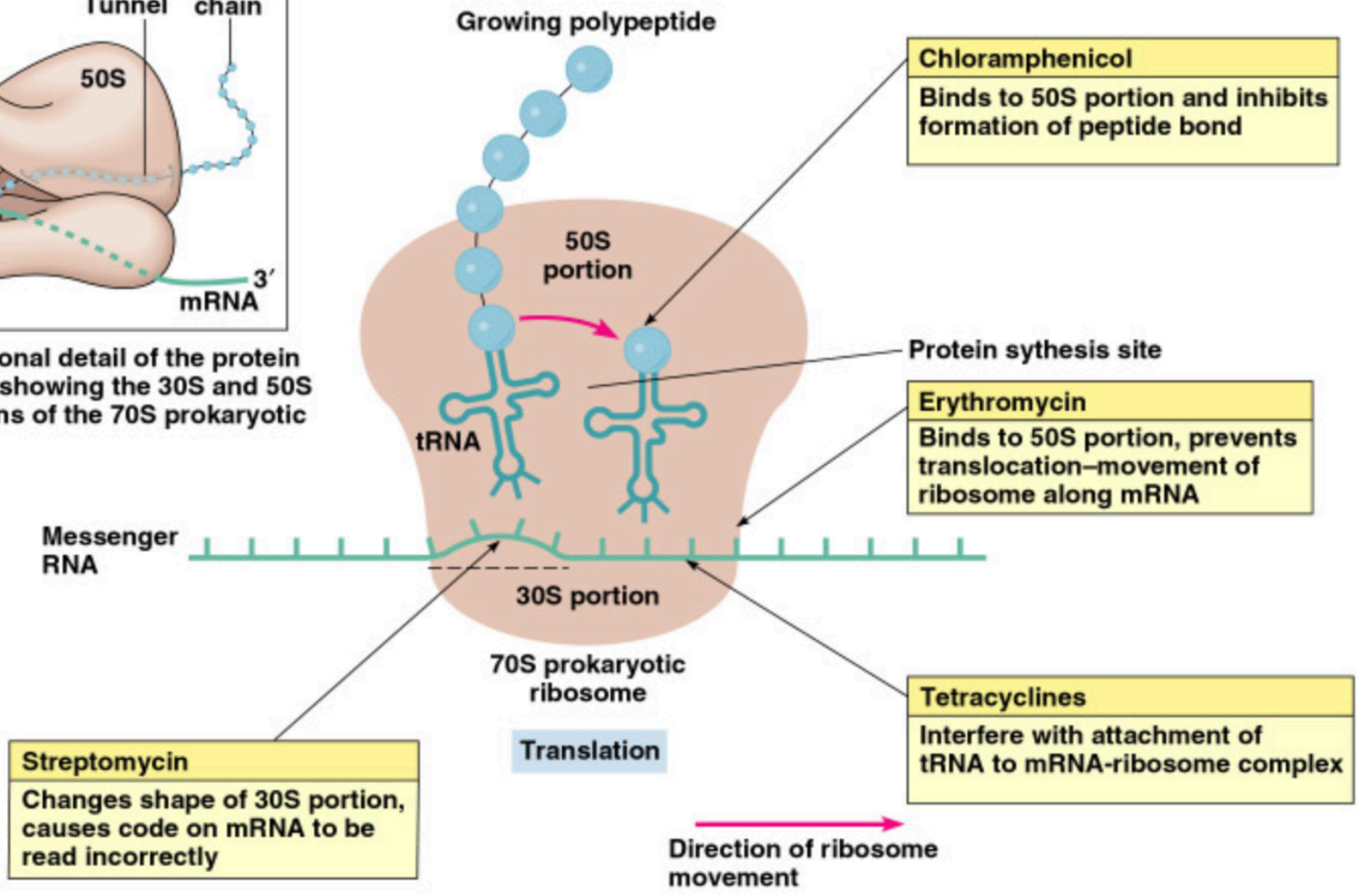

undecaprenyl-pyrophosphate
N-acetyl glucosamine
N-acetyl muramic acid
L-glycine
D-alanine
L-lysine
D-glutamic acid
L-alanine
potassium ion
pentaglycine crosslinking bridge
lysostaphin
PBPs
oxacillin
transpeptidation
D-ala-D-ala
vancomycin
teicoplanin
transglycosylation (PBP2)
flavomycin
lipid carrier recycling
(pyrophosphorylase)
bacitracin
membrane depolarization
daptomycin
K+
membrane
(MurA)
fosfomycin
(MraY)
tunicamycin
UMP
UDP
PEP NADPH
L-Ala
D-Glu
L-Lys
D-Ala2
tRNAGly 2 x tRNAGly 2 x tRNAGly
D-alanine ligase
D-alanine racemase
D-cycloserine
2 x D-Ala
L-Ala

Antimicrobianos que Inibem a Síntese Protéica



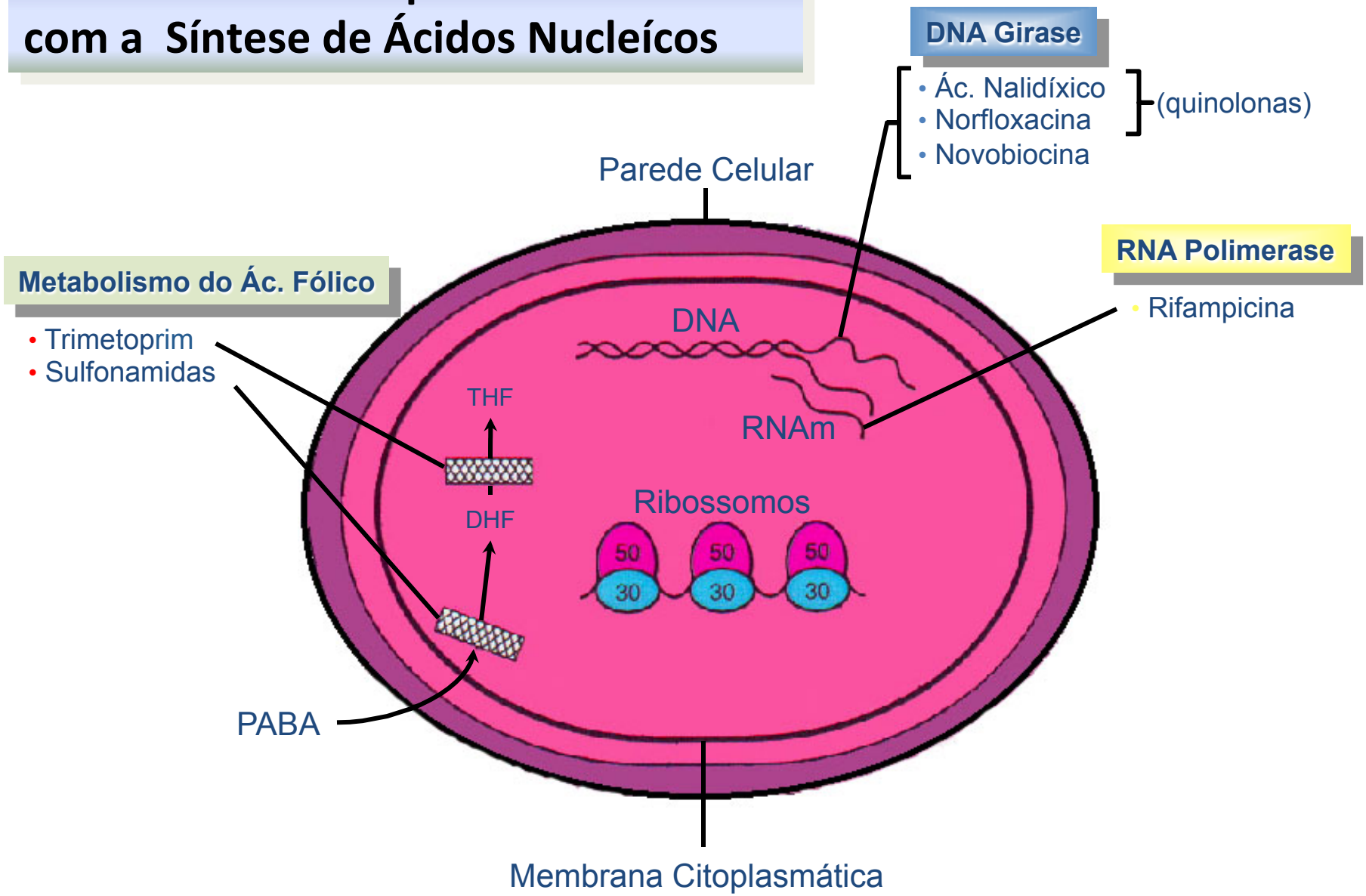


(a) Three-dimensional detail of the protein synthesis site showing the 30S and 50S subunit portions of the 70S prokaryotic ribosome.

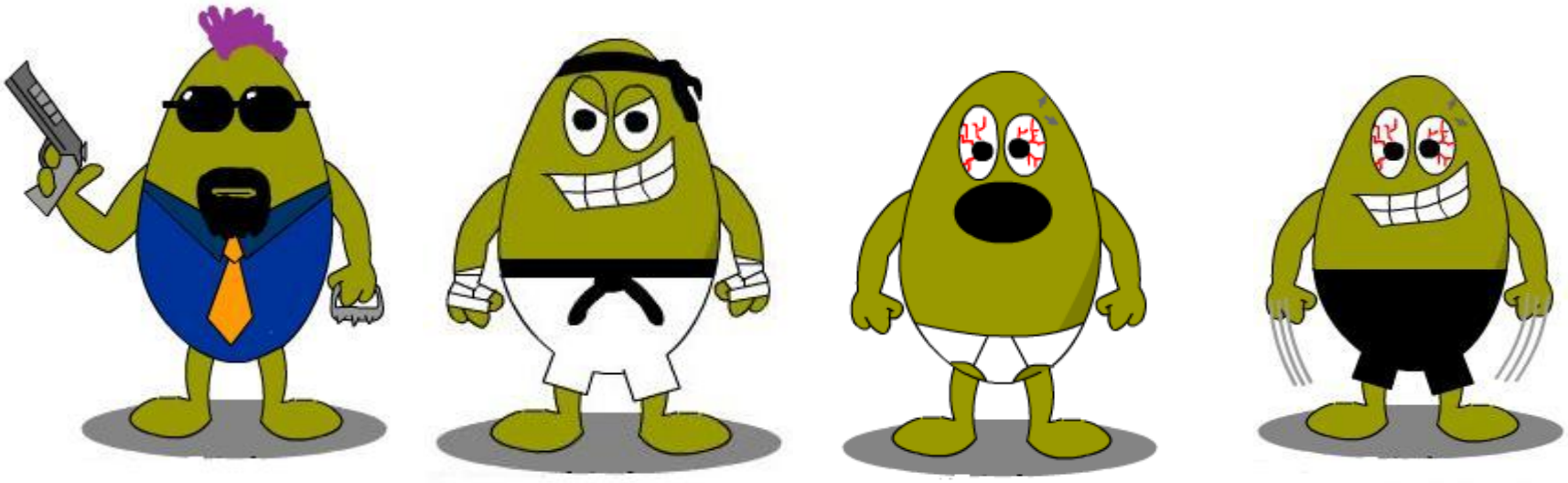


(b) In the diagram the black arrows indicate the different points at which chloramphenicol, erythromycin, the tetracyclines, and streptomycin exert their activities.

Antibmicrobianos que Interferem com a Síntese de Ácidos Nucleícos



Mecanismos de Resistência a Antimicrobianos



Origem da Resistência a Antimicrobianos

Aspectos relacionados à resistência aos antimicrobianos em bactérias

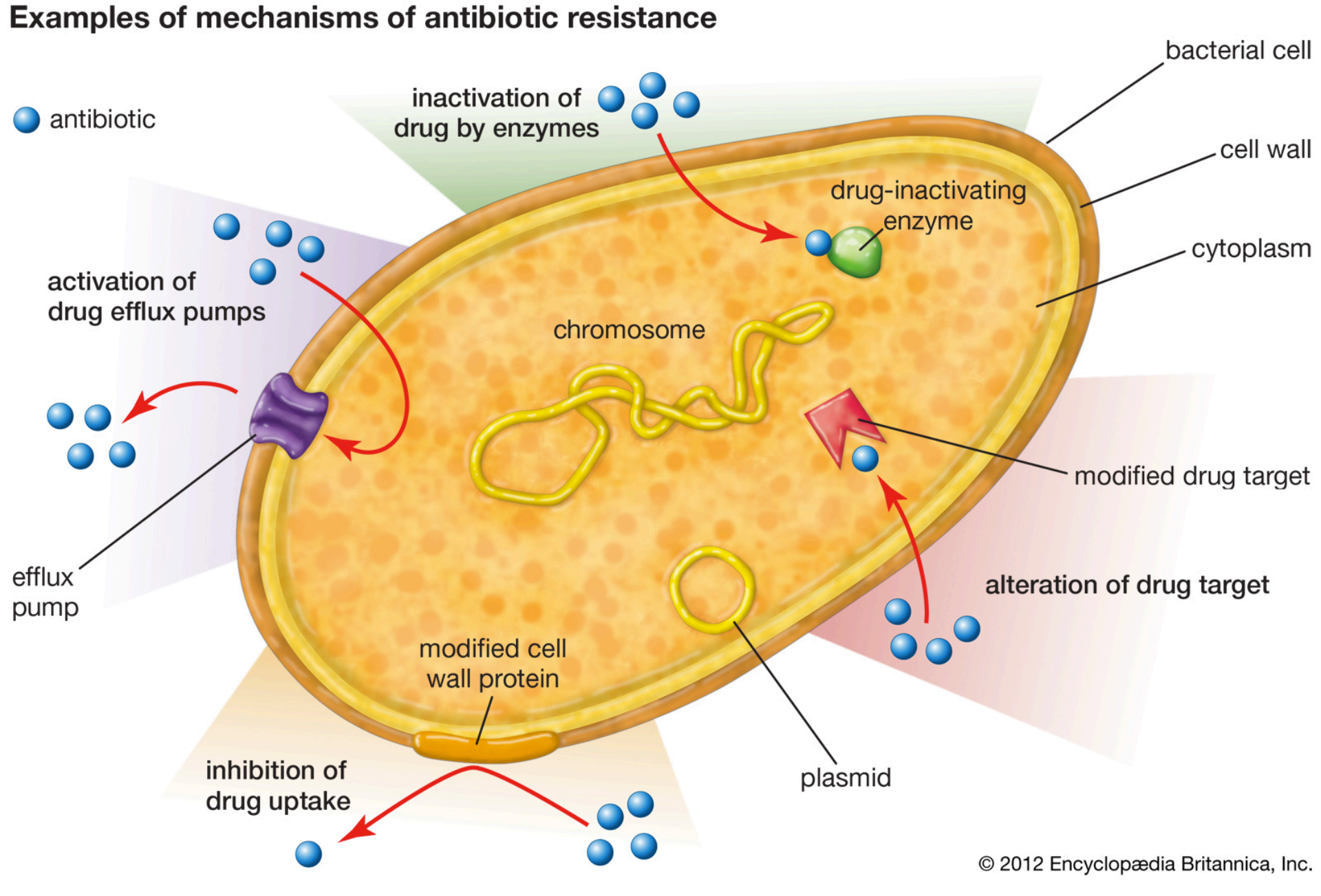
★ Origem da Resistência

- ✦ resistência cromossomal
- ✦ resistência extracromossomal

★ Mecanismos de transmissão

- ✦ transmissão vertical
- ✦ transmissão horizontal

➢ Resistência Intrínseca e Extrínseca


Examples of mechanisms of antibiotic resistance
antibiotic
inactivation of drug by enzymes
bacterial cell
cell wall
drug-inactivating enzyme
cytoplasm
activation of drug efflux pumps
chromosome
modified drug target
efflux pump
alteration of drug target
modified cell wall protein
plasmid
inhibition of drug uptake
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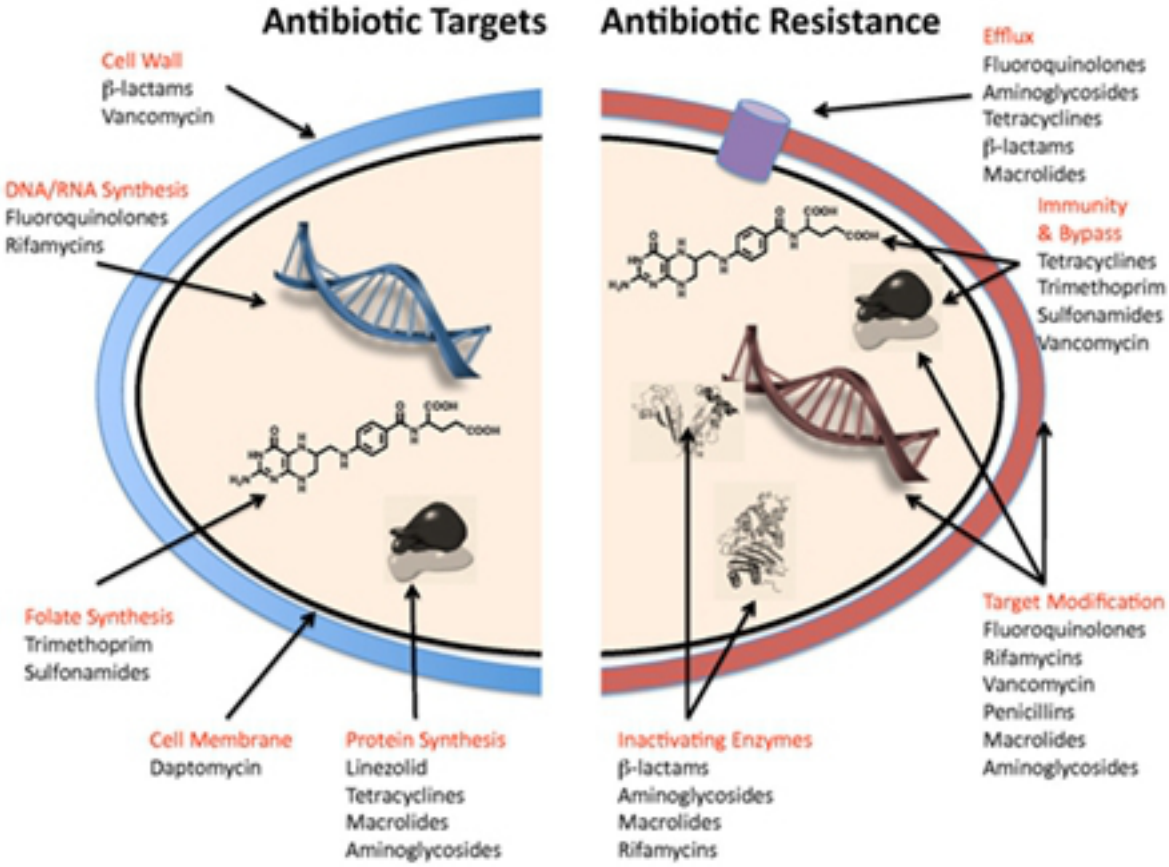

Antibiotic Targets
Antibiotic Resistance
Cell Wall
β-lactams
Vancomycin
DNA/RNA Synthesis
Fluoroquinolones
Rifamycins
Folate Synthesis
Trimethoprim
Sulfonamides
Cell Membrane
Daptomycin
Protein Synthesis
Linezolid
Tetracyclines
Macrolides
Aminoglycosides
Efflux
Fluoroquinolones
Aminoglycosides
Tetracyclines
β-lactams
Macrolides
Immunity
& Bypass
Tetracyclines
Trimethoprim
Sulfonamides
Vancomycin
Target Modification
Fluoroquinolones
Rifamycins
Vancomycin
Penicillins
Macrolides
Aminoglycosides
Inactivating Enzymes
β-lactams
Aminoglycosides
Macrolides
Rifamycins

Mecanismos de Resistência a Antimicrobianos

- Alteração do alvo
- Enzimas inativadoras ou modificadoras
- Bombas de efluxo
- Impermeabilidade do envoltório celular

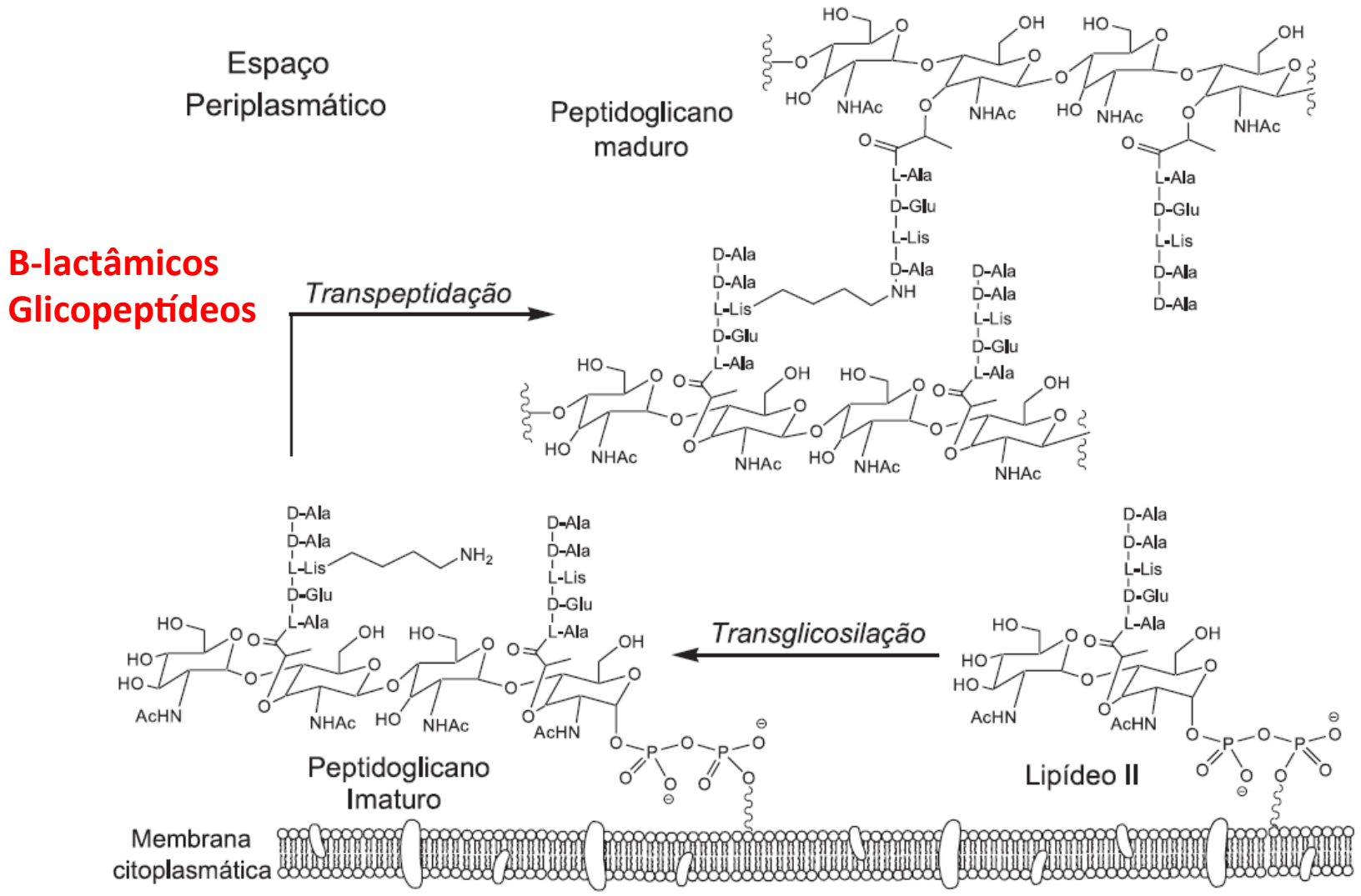
1 – Alteração do alvo

- Por alteração da sequência de aminoácidos da proteína alvo;
- Por modificação química do alvo mediada por enzimas.

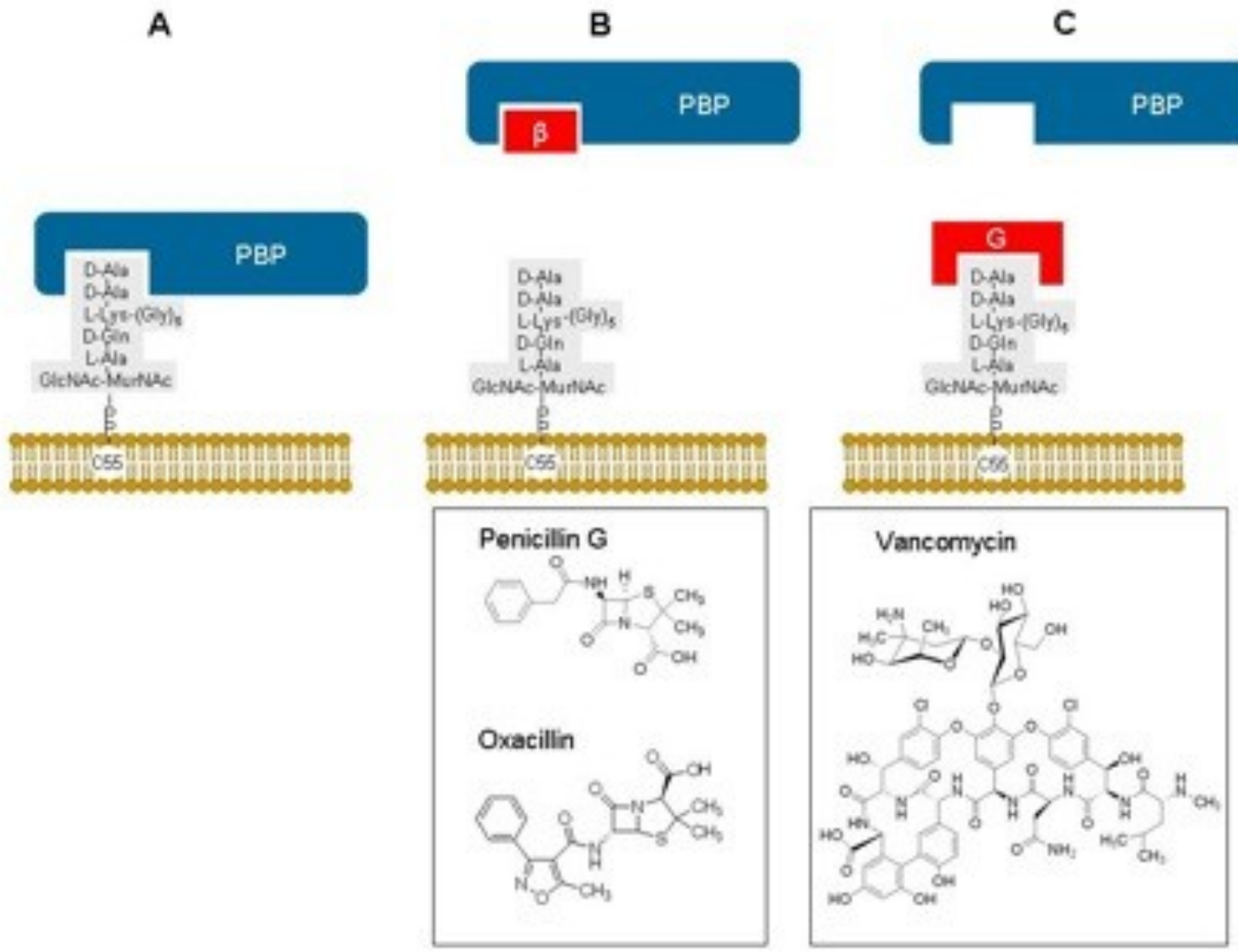
★ Exemplos:

- ✦ **β-lactâmicos**
- ✦ **Glicopeptídeos**
- ✦ **Sulfonamidas**
- ✦ **Rifampicina**
- ✦ **Quinolonas**
- ✦ **Aminoglicosídeos**

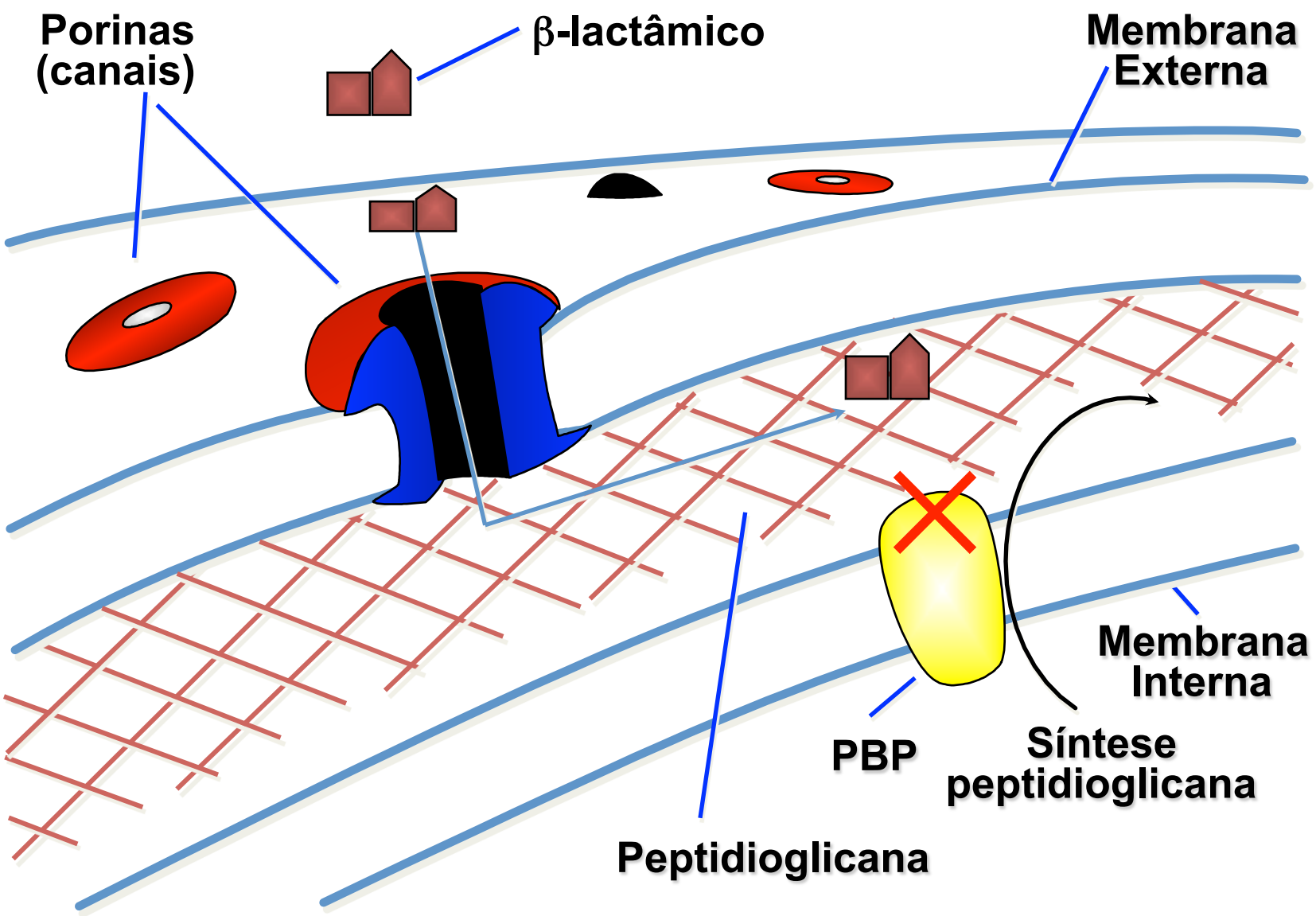
Formação do Peptideoglicano e Ação de Antimicrobianos que Agem na Parede Celular



Formação do Peptideoglicano e Ação de Antibióticos que Agem na Parede Celular

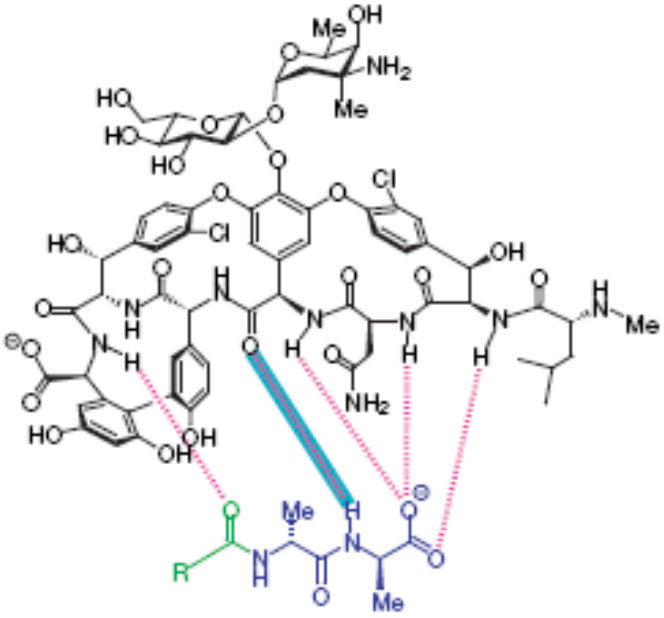


Resistência Mediada por Alteração de Alvo

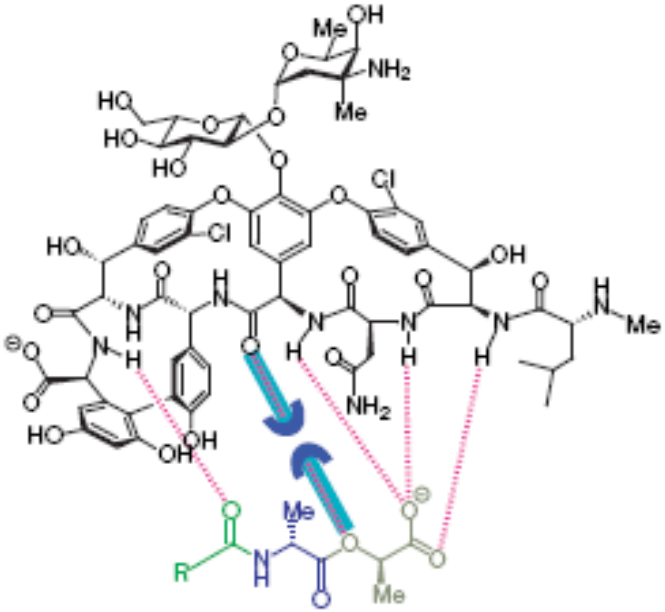


Resistência Mediada por Alteração de Alvo

Glicopeptídeo:
Vancomicina



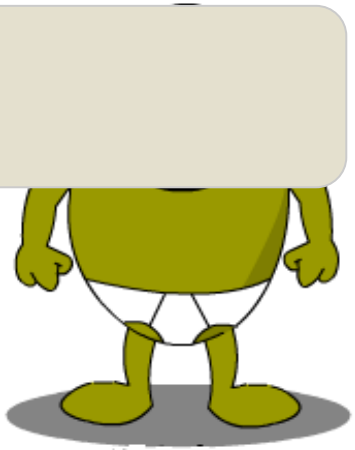
N-acyl-D-Ala-D-Ala



N-acyl-D-Ala-D-Lac

- *Enterococcus* resistente à vancomicina (VRE)

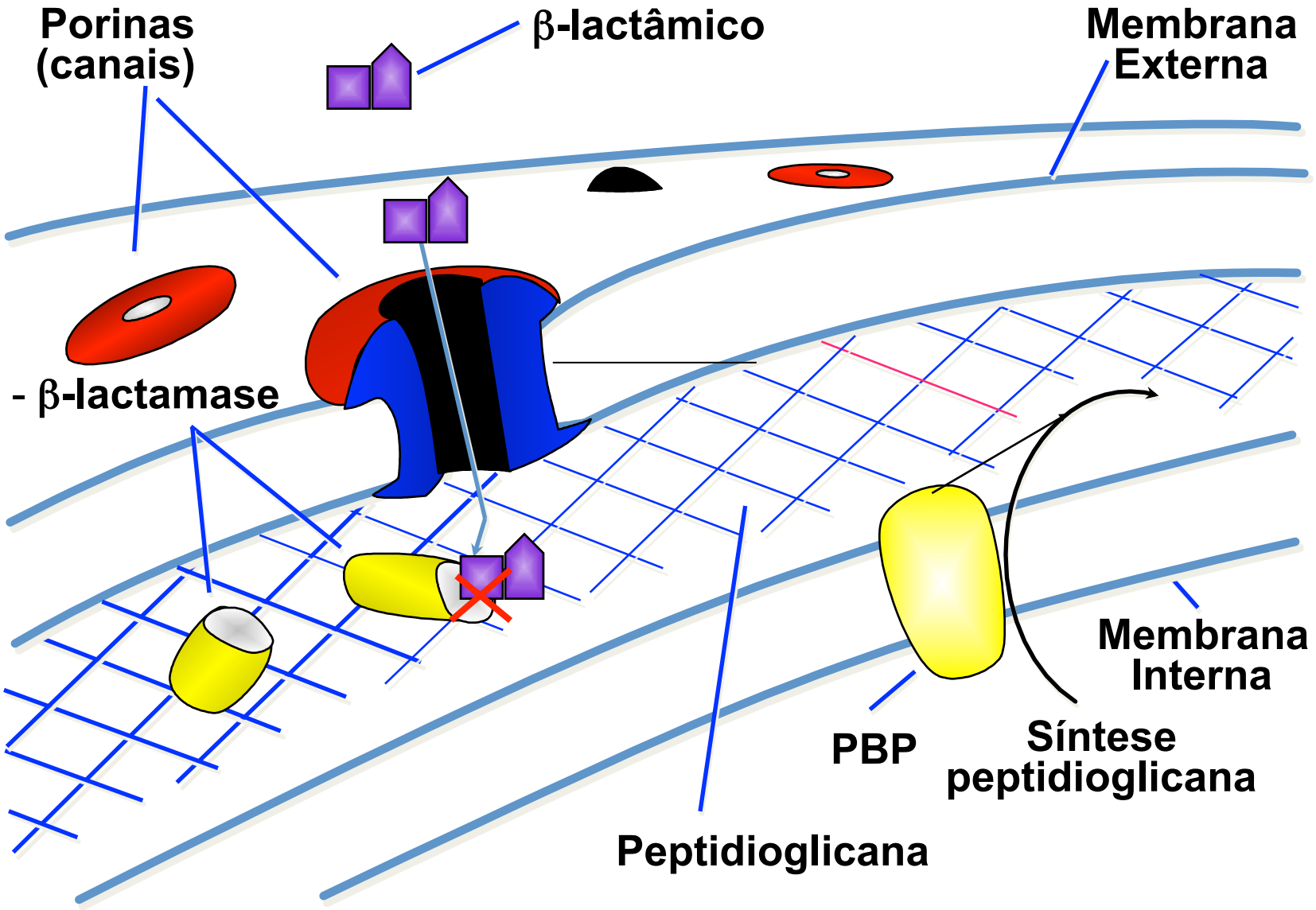
2 – Enzimas inativadoras



★ Exemplos:

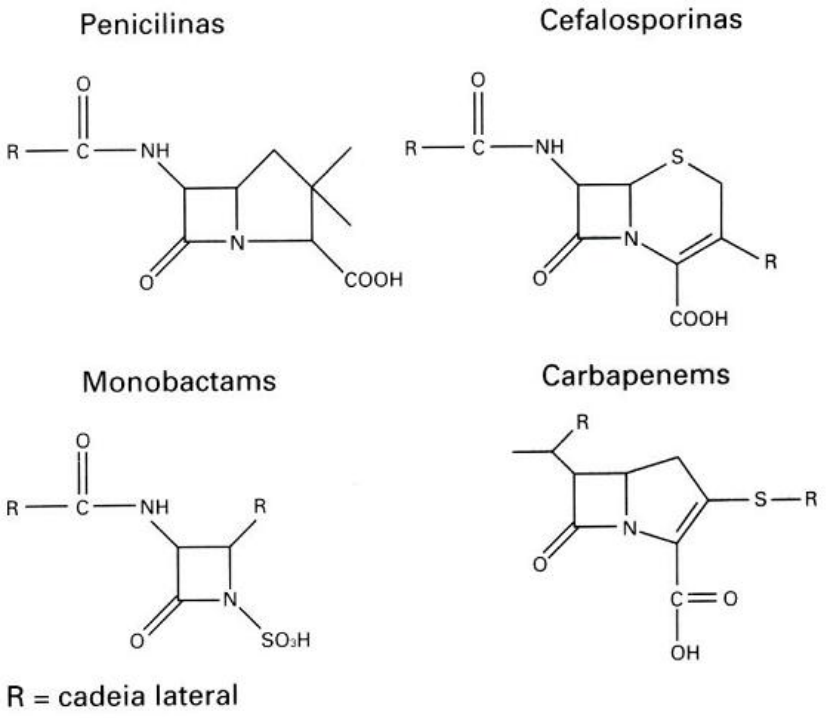
- ✦ β-lactâmicos
- ✦ Aminoglicosídeos
- ✦ Clorafenicol

Resistência Mediada por β-lactamases



Resistência Mediada por Enzimas Inativadoras (hidrólise)

β-lactâmicos



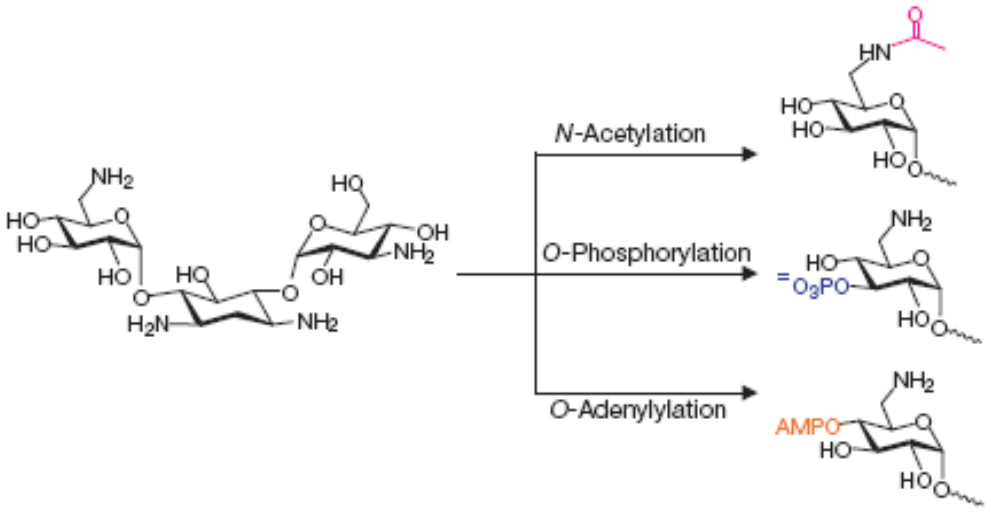
β-lactamases

- Classe A ou grupo II: hidrolisam penicilinas e cefalosporinas
- Classe B ou grupo III: hidrolisam carbapenêmicos
- Classe C ou grupo I: hidrolisam cefalosporinas
- Classe D: hidrolisam penicilinas e cloxacilina
- Grupo IV: hidrolisam penicilina

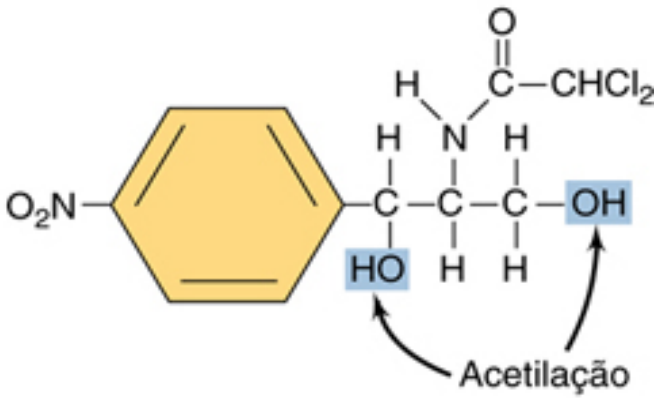
IMPORTANTE: β-lactamases de amplo espectro ou espectro estendido (ESBL – Extended Spectrum β-lactamases)

Resistência Mediada por Enzimas Inativadoras

Aminoglicosídeos



Clorafenicol



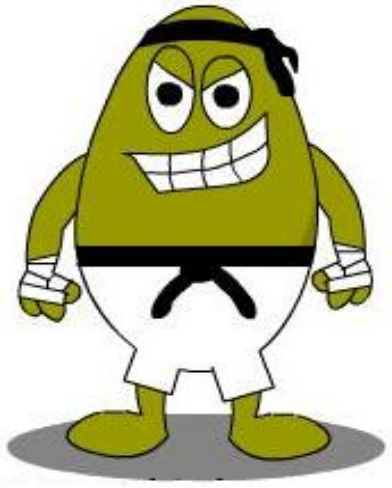
3 – alteração na permeabilidade da membrana

★ Exemplos:

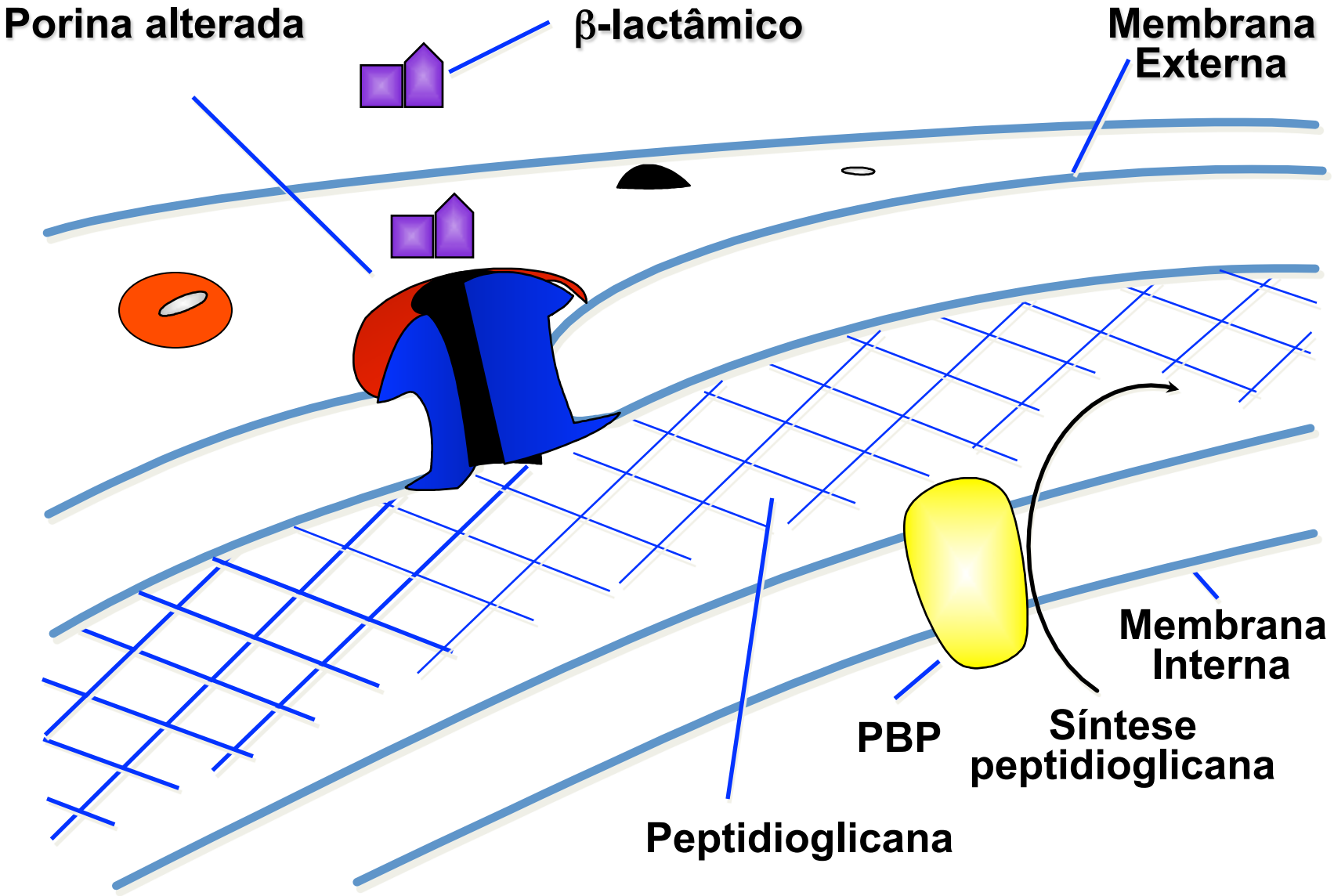
β-lactâmicos

Aminoglicosídeos

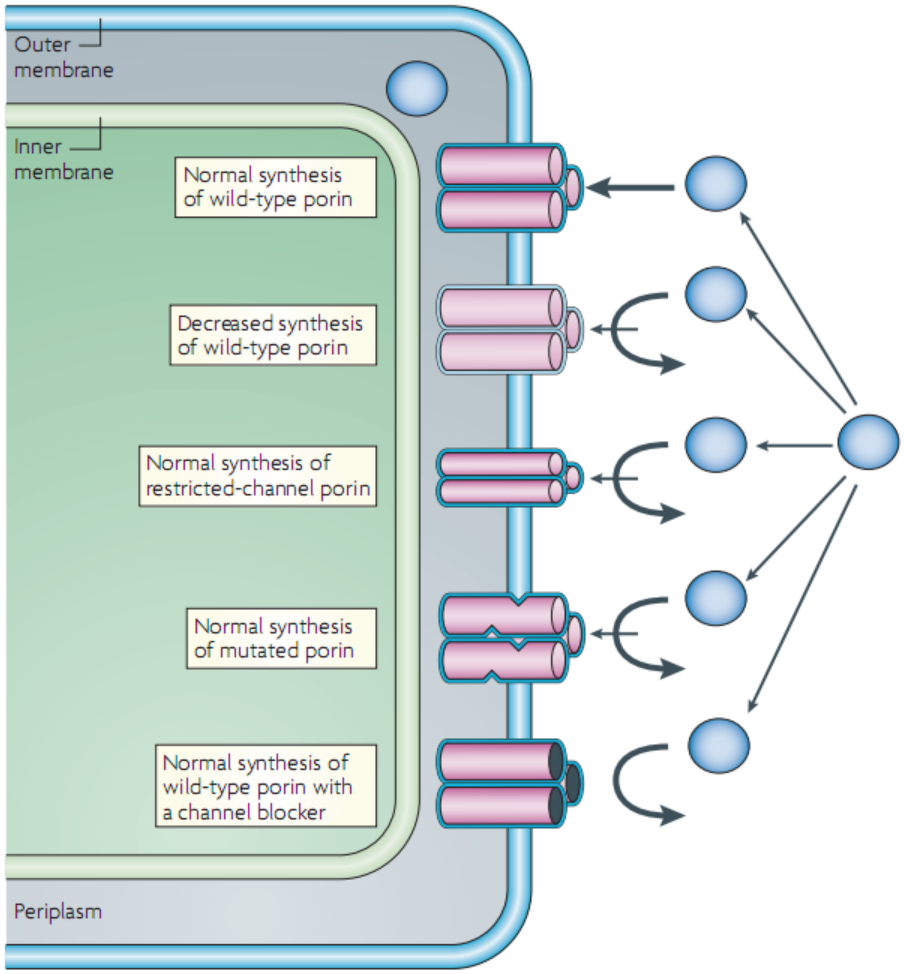
Quinolonas



Resistência mediada por alteração de permeabilidade



Resistência mediada por alteração de permeabilidade



Diminuição da expressão de OmpF leva a resistência a:

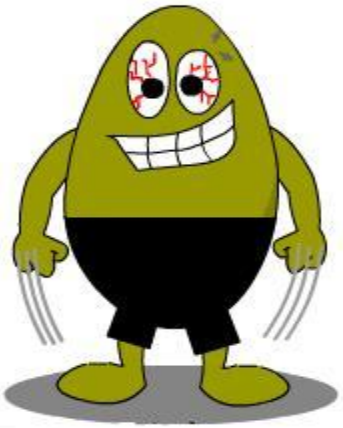
quinolonas

tetraciclinas

clorafenicol

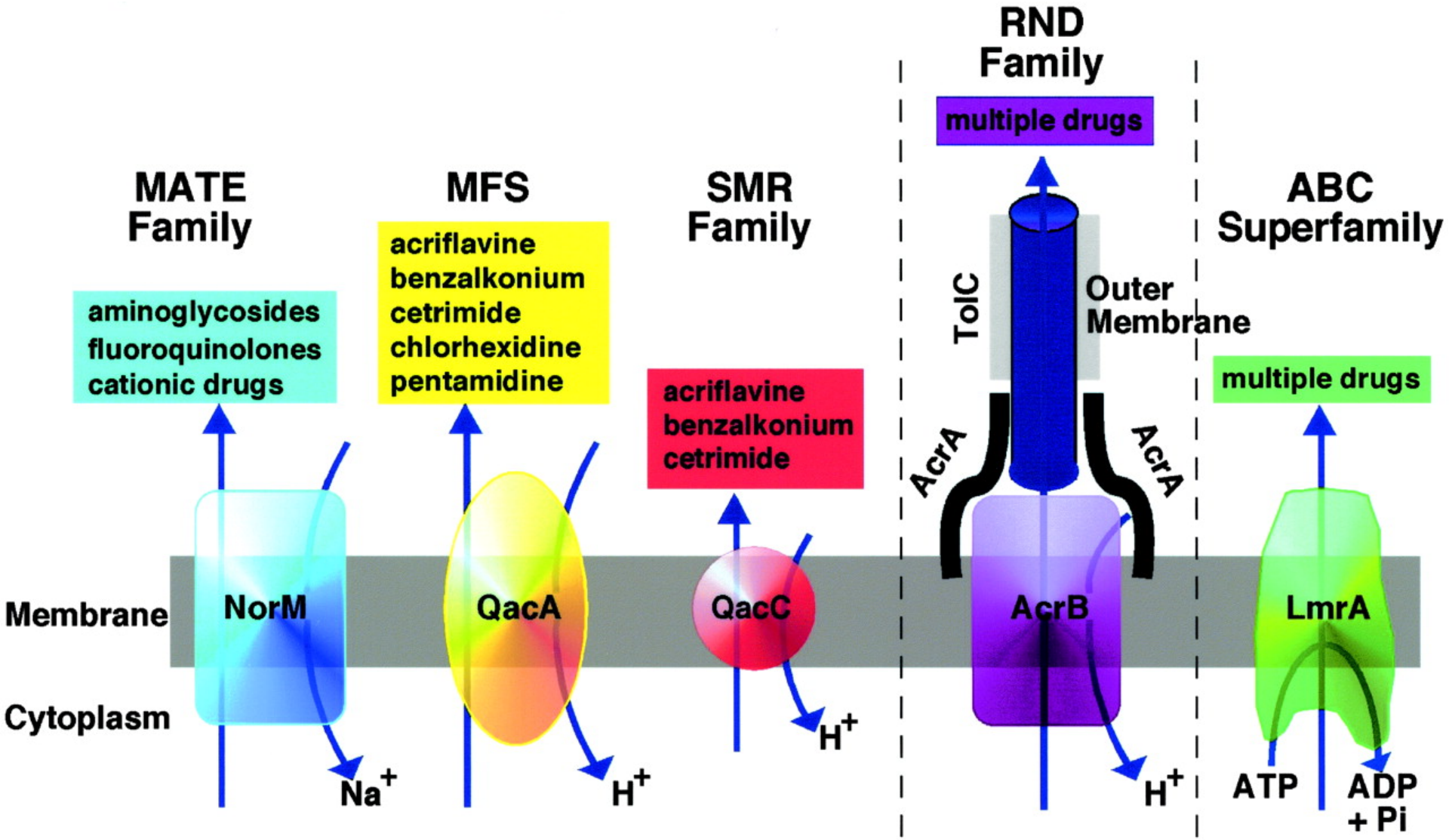
β-lactâmicos

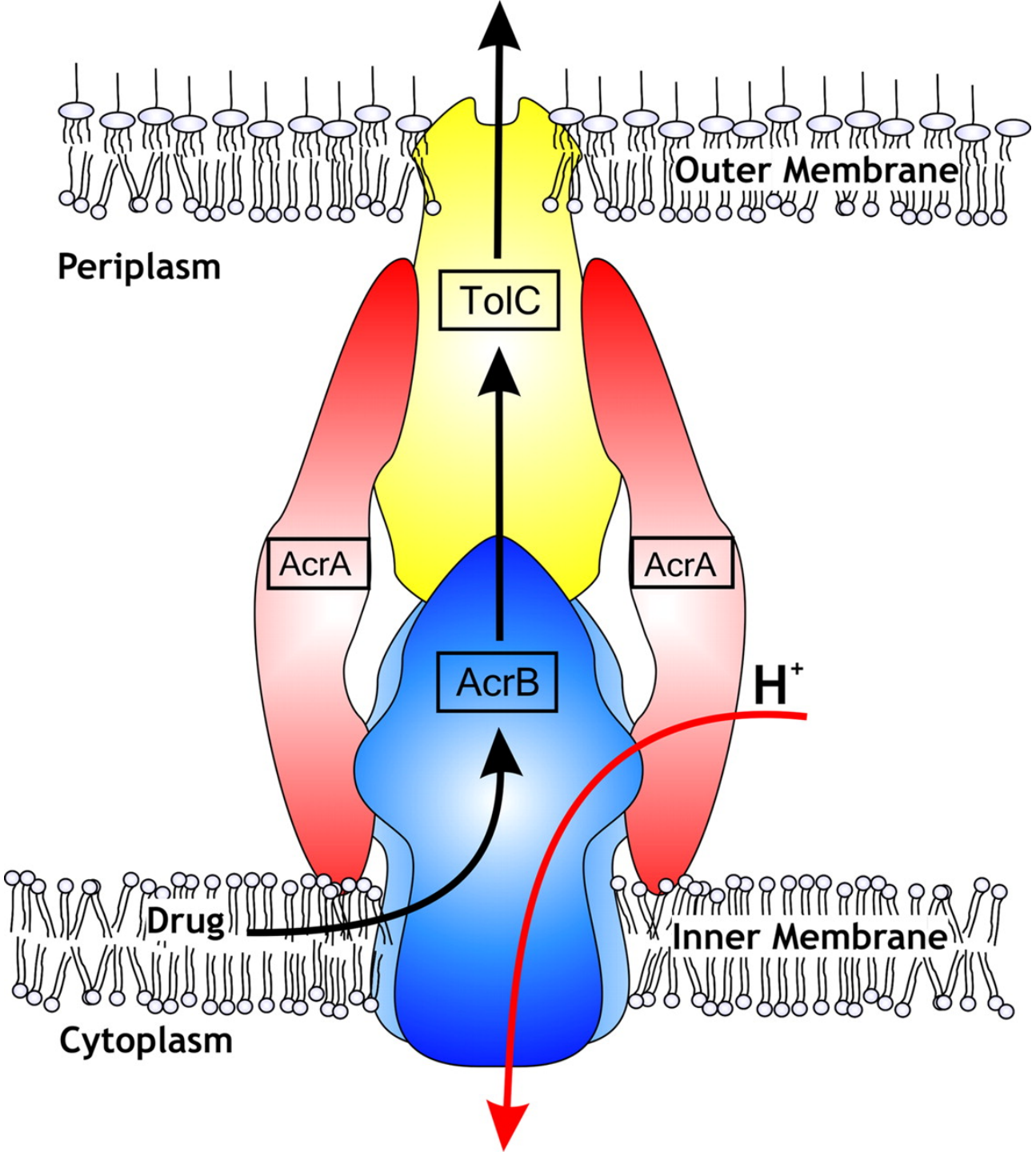
4 . Efluxo



★ Exemplos:

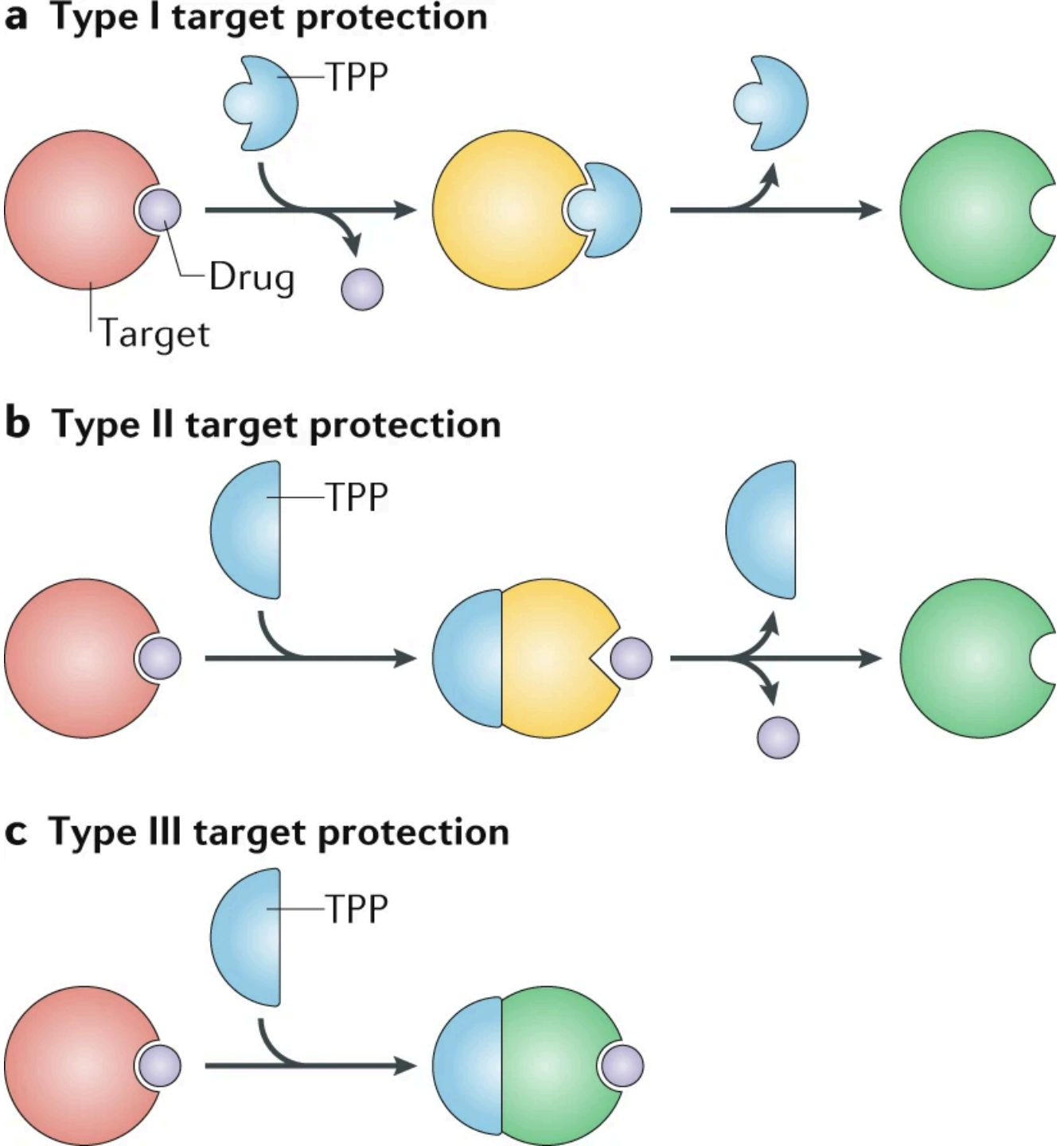
- ✦ Tetraciclina
- ✦ β-lactâmicos
- ✦ Cloranfenicol
- ✦ Quinolonas


RND
Family
multiple drugs
MATE
Family
MFS
SMR
Family
ABC
Superfamily
aminoglycosides
fluoroquinolones
cationic drugs
acriflavine
benzalkonium
cetrimide
chlorhexidine
pentamidine
acriflavine
benzalkonium
cetrimide
TolC
Outer
Membrane
AcrA
AcrA
multiple drugs
Membrane
Cytoplasm
NorM
QacA
QacC
AcrB
LmrA
Na+
H+
H+
H+
ATP
ADP
+ Pi


Outer Membrane
Periplasm
TolC
AcrA
AcrA
AcrB
H+
Drug
Inner Membrane
Cytoplasm

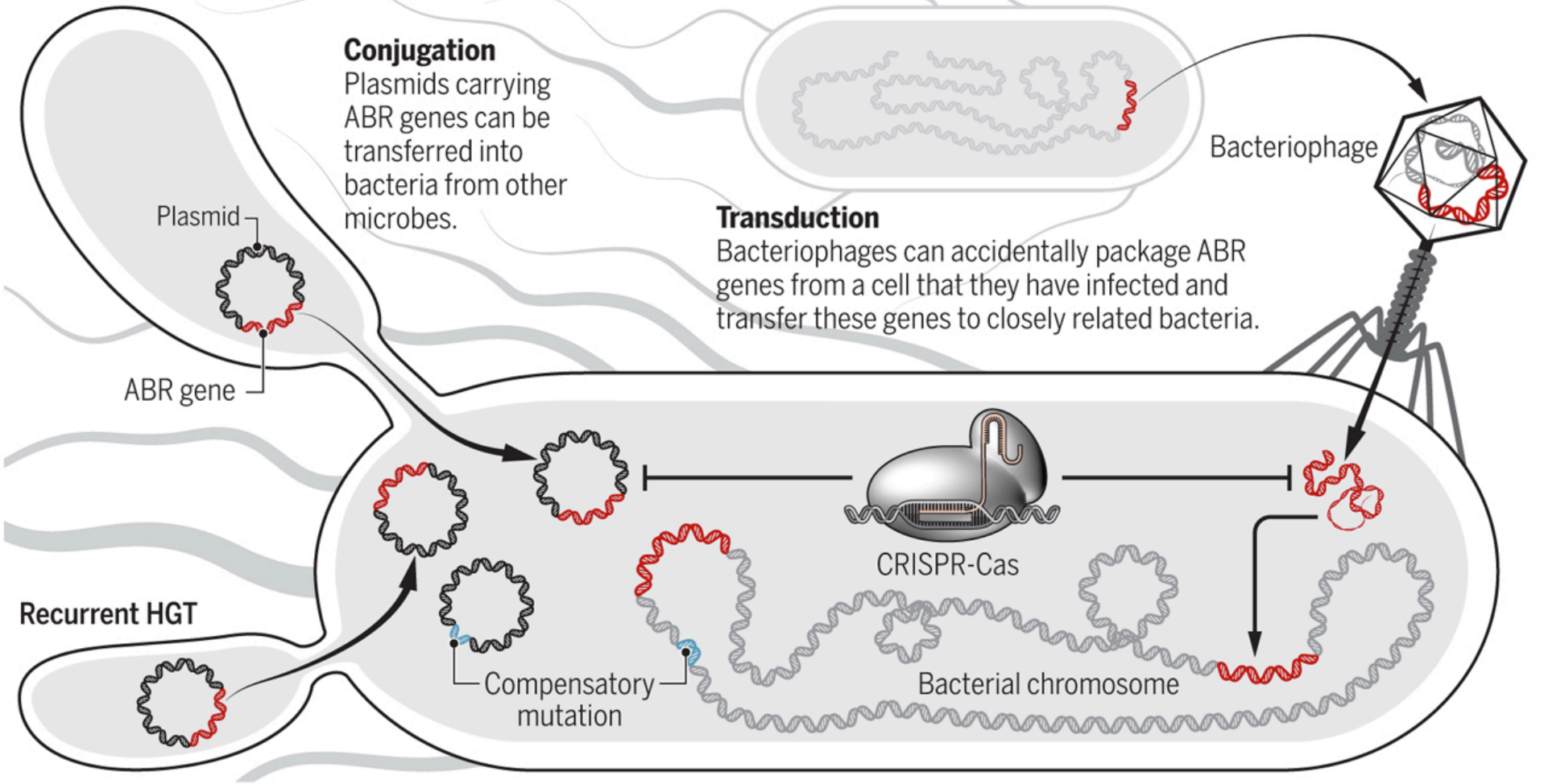
Target Protection Proteins TPP

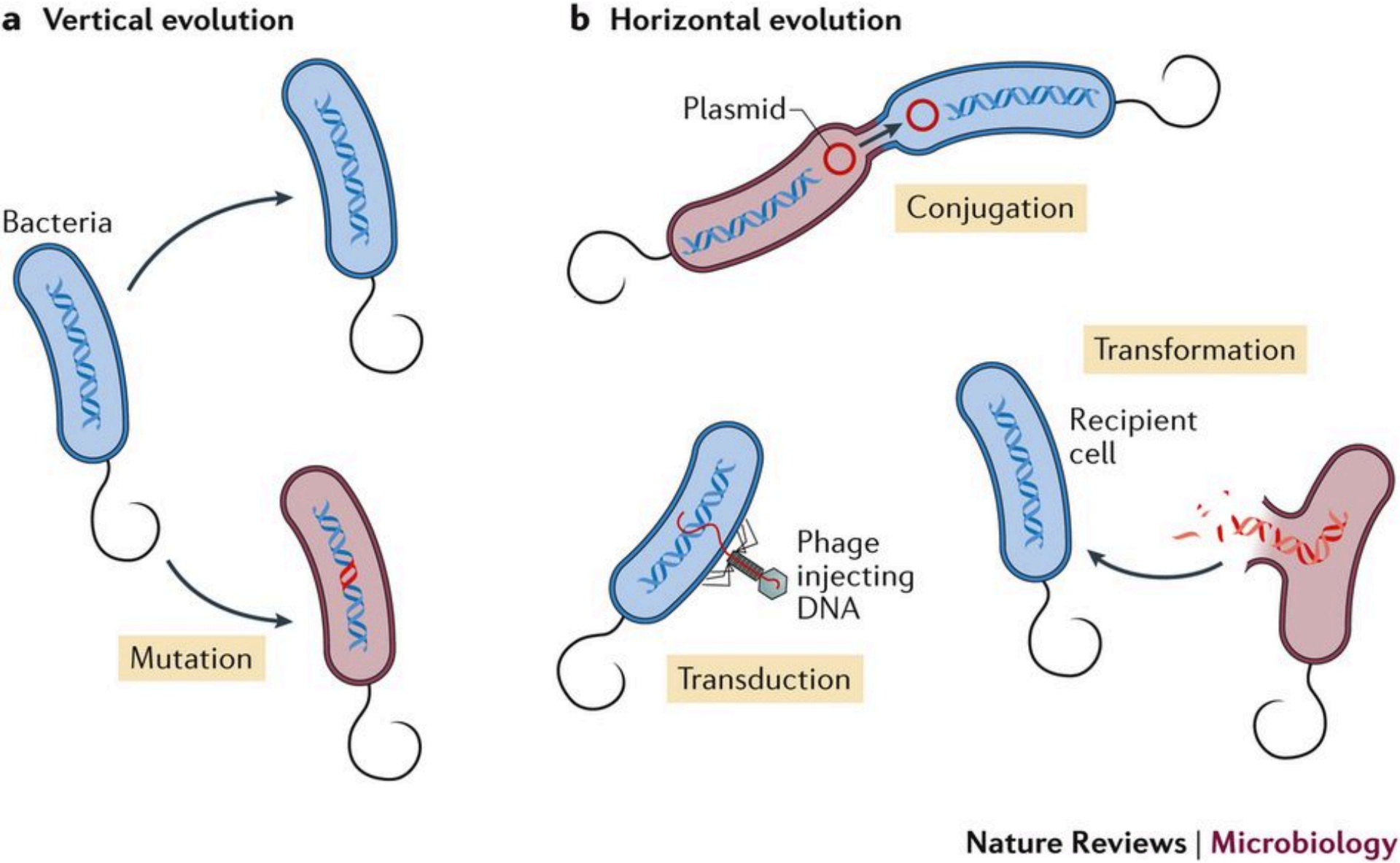
Target protection proteins TPP



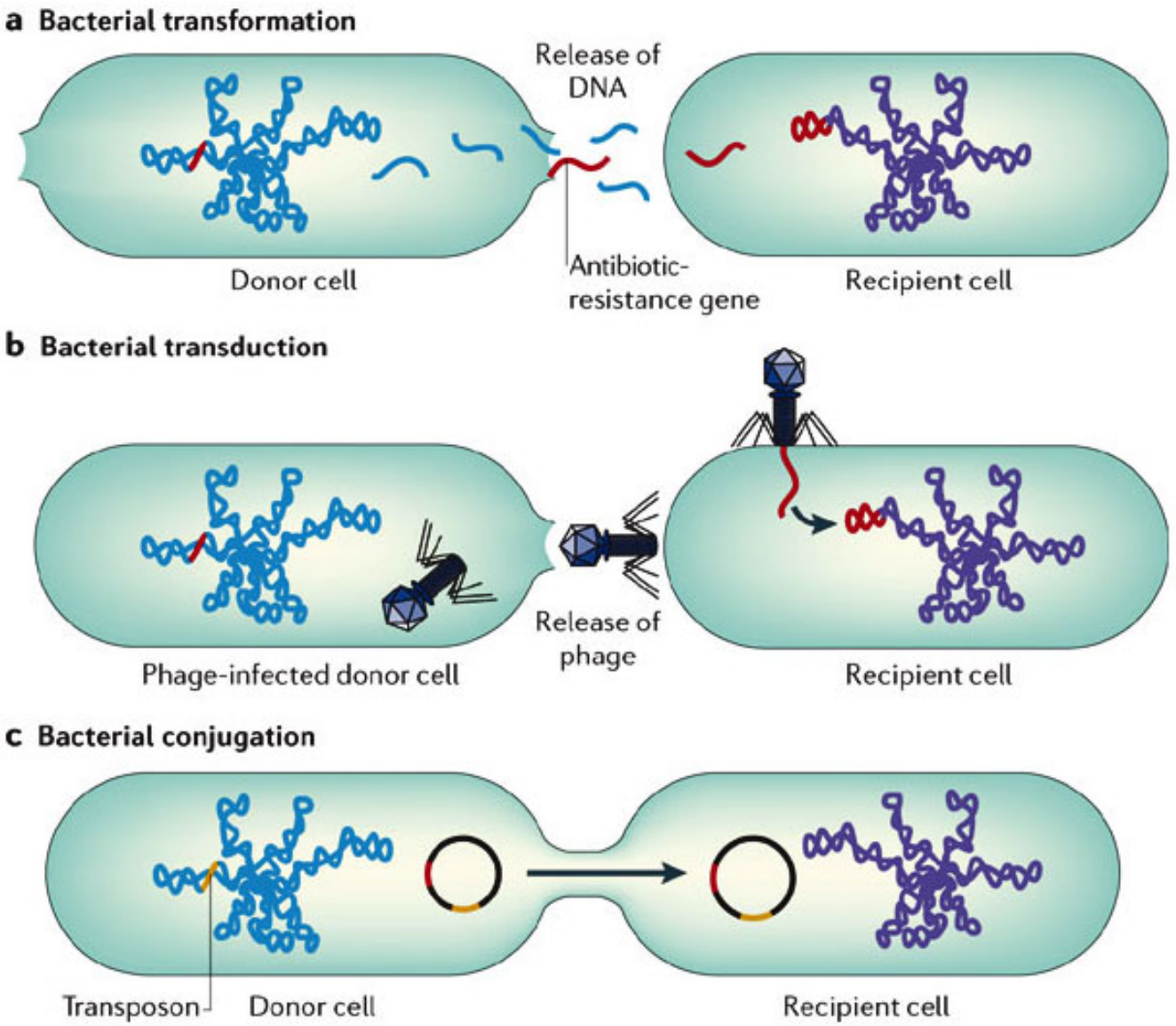
Mechanisms of mobile antibiotic resistance

Pathogenic bacteria can acquire antibiotic resistance (ABR) genes through two main mechanisms of horizontal gene transfer (HGT): conjugation and transduction. Compensatory mutations alleviate the fitness costs imposed by ABR genes, contributing to their stabilization. CRISPR-Cas–based systems may selectively destroy DNA carrying mobile ABR genes.



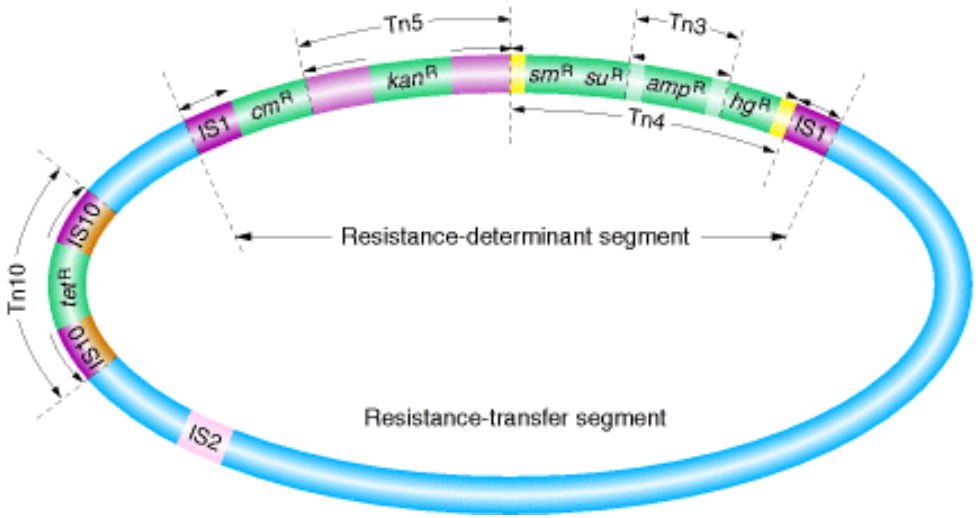

a Vertical evolution
Bacteria
Mutation
b Horizontal evolution
Plasmid
Conjugation
Transformation
Recipient cell
Phage injecting DNA
Transduction

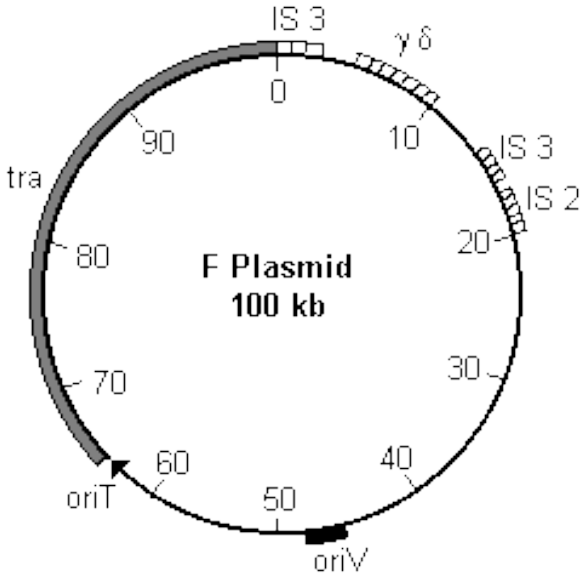
Transmissão Horizontal



Plamídeos de Resistência

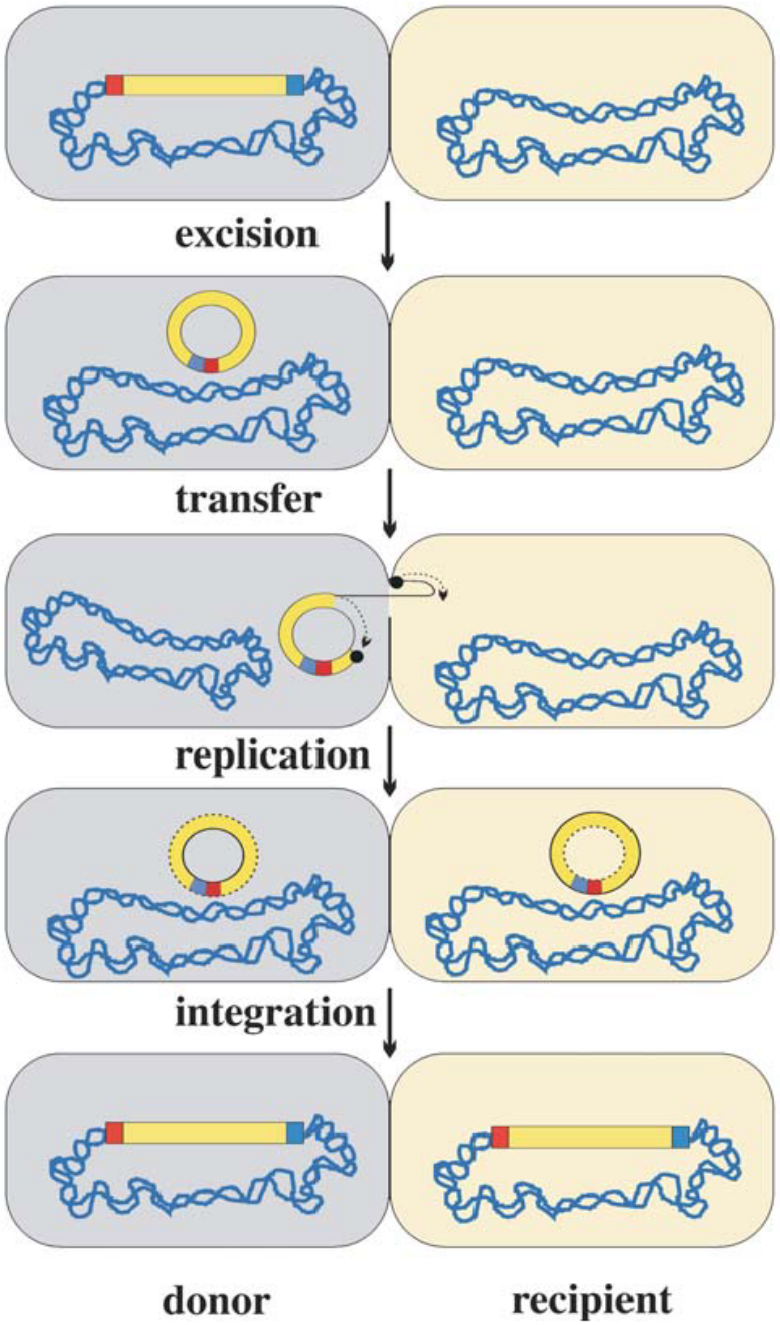
- Carregam genes de resistência
- Podem conter transposons e/ou integrons
- Podem ser transferidos (geralmente através de conjugação), mas também por transformação ou transdução

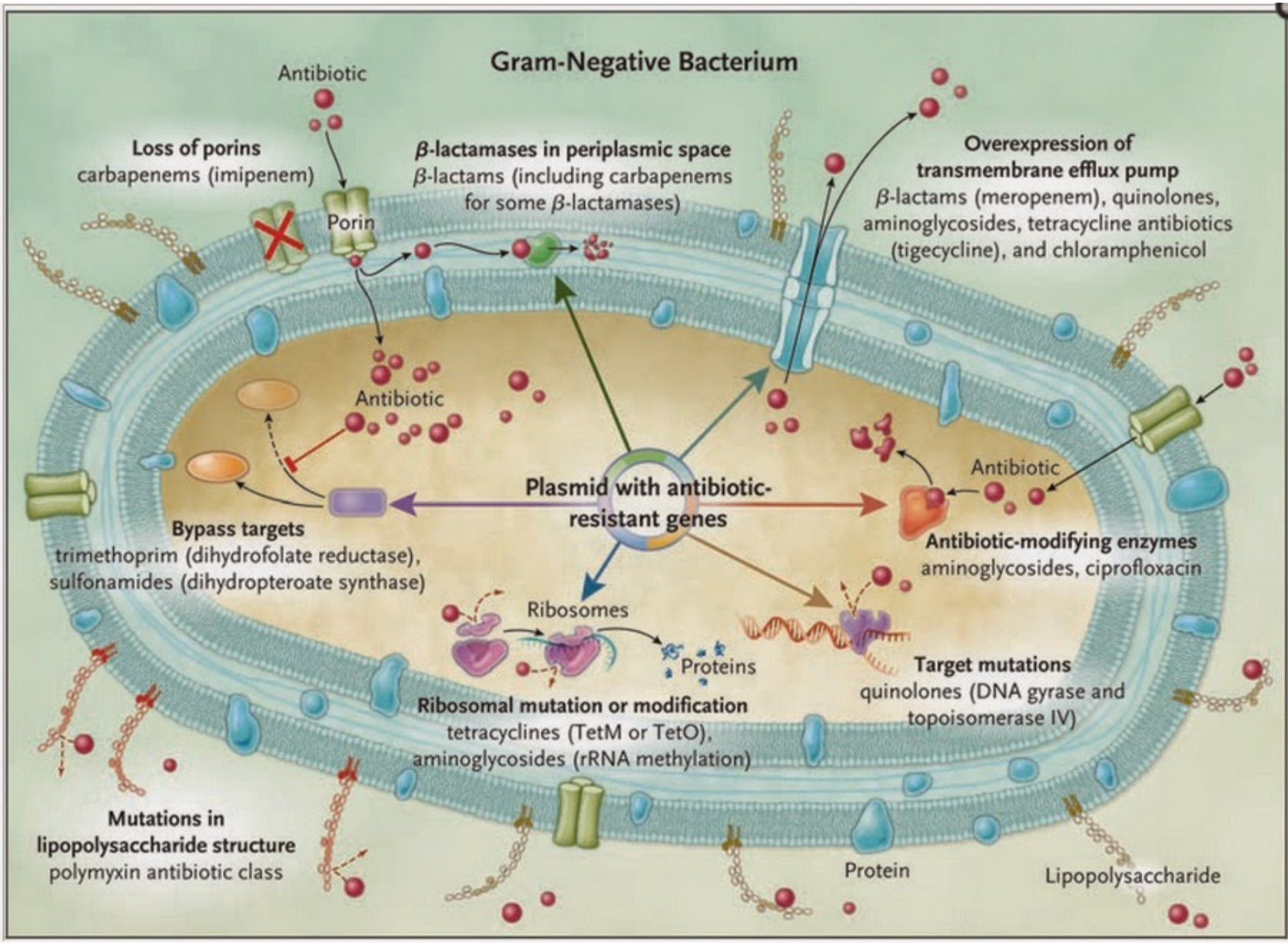




Transposons Conjugativos

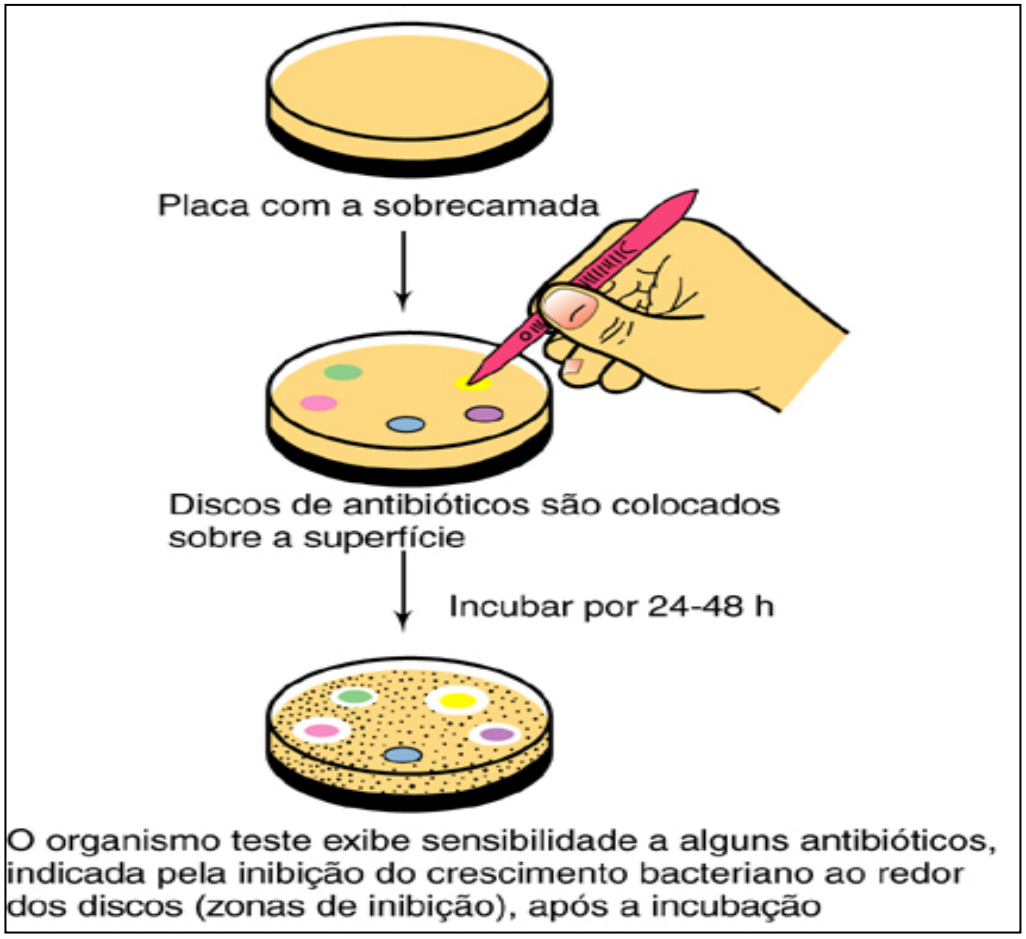
Mecanismo de transferência




Gram-Negative Bacterium
Antibiotic
Loss of porins
carbapenems (imipenem)
Porin
β-lactamases in periplasmic space
β-lactams (including carbapenems
for some β-lactamases)
Overexpression of
transmembrane efflux pump
β-lactams (meropenem), quinolones,
aminoglycosides, tetracycline antibiotics
(tigecycline), and chloramphenicol
Antibiotic
Antibiotic
Plasmid with antibiotic-
resistant genes
Bypass targets
trimethoprim (dihydrofolate reductase),
sulfonamides (dihydropteroate synthase)
Antibiotic-modifying enzymes
aminoglycosides, ciprofloxacin
Ribosomes
Proteins
Target mutations
quinolones (DNA gyrase and
topoisomerase IV)
Ribosomal mutation or modification
tetracyclines (TetM or TetO),
aminoglycosides (rRNA methylation)
Mutations in
lipopolysaccharide structure
polymyxin antibiotic class
Protein
Lipopolysaccharide

Método de Medição da Resistência Bacteriana

Métodos de Medição de Resistência

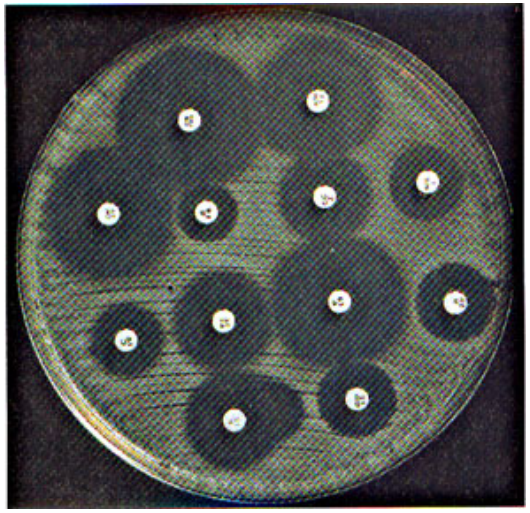


O organismo teste exibe sensibilidade a alguns antibióticos, indicada pela inibição do crescimento bacteriano ao redor dos discos (zonas de inibição), após a incubação

Métodos de Medição de Resistência

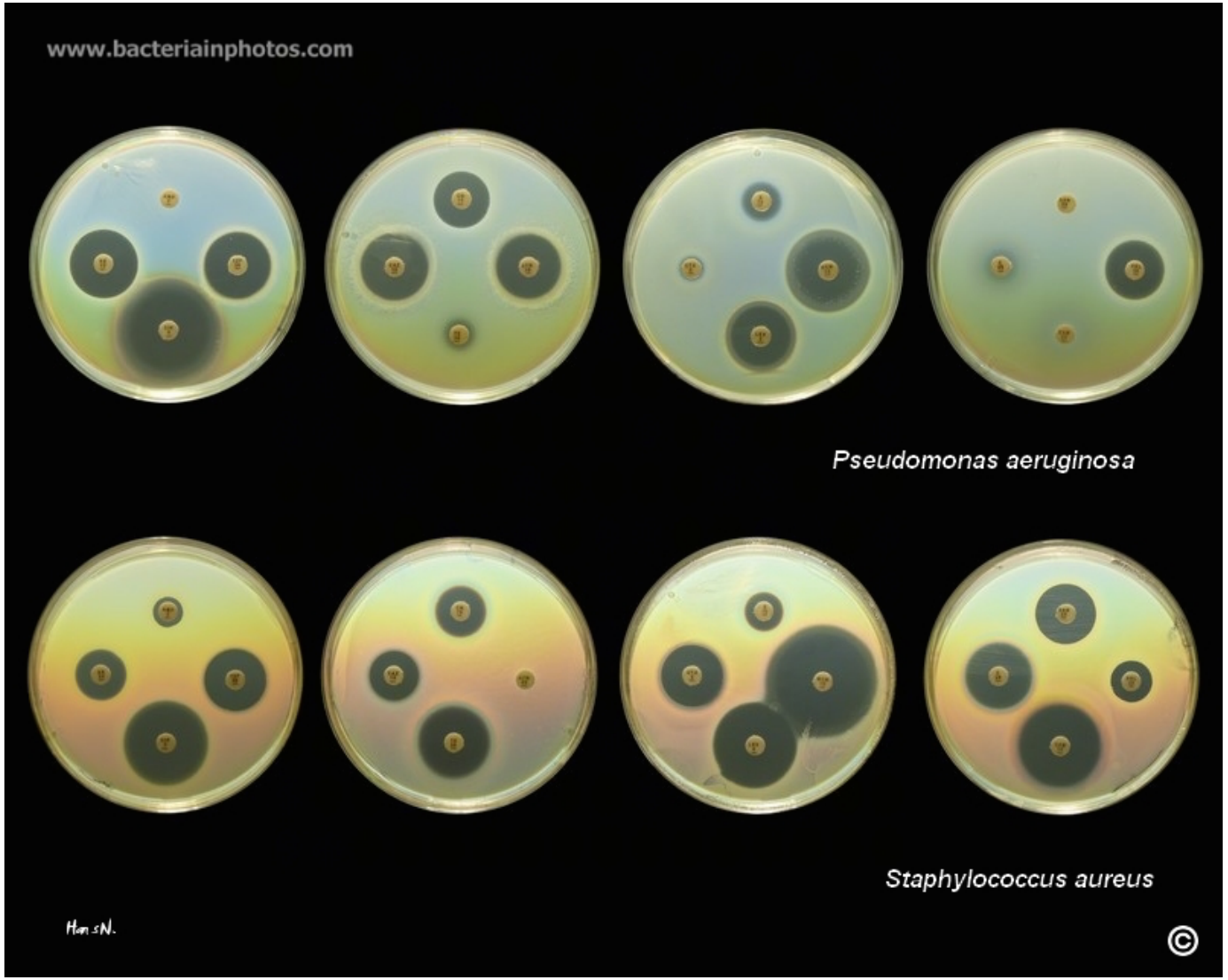
- **Método qualitativo:** Método de difusão com discos de Kirby-Bauer
- **Método quantitativo:** Macrodiluição.

Antibiograma



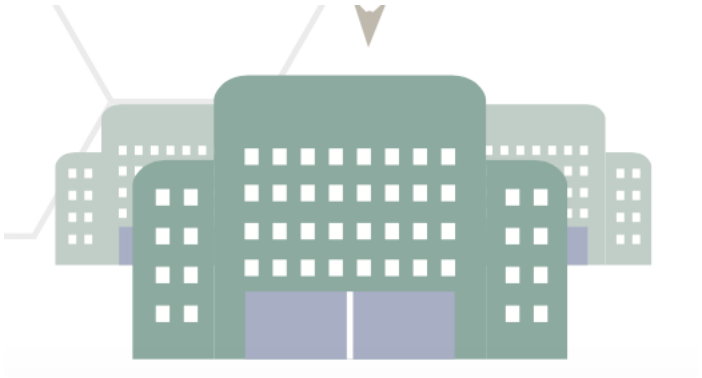
Macrodiluição

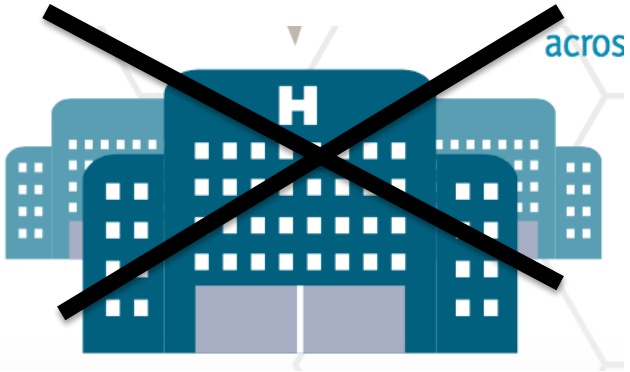
Teste de difusão em disco de ágar



Plano de iniciativas para vencer a resistência

- **Estabelecimento de Programas de Prevenção, grupos dedicados à educação em saúde, prevenir infecções**

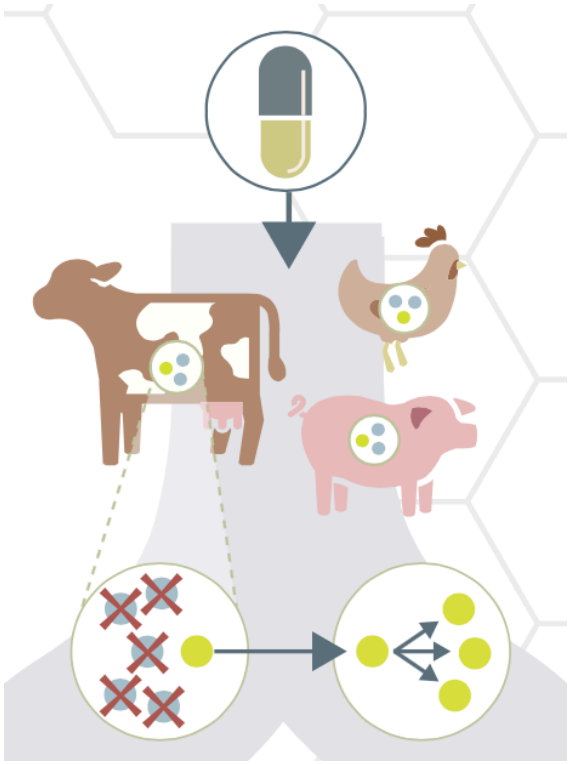




Plano de iniciativas para vencer a resistência

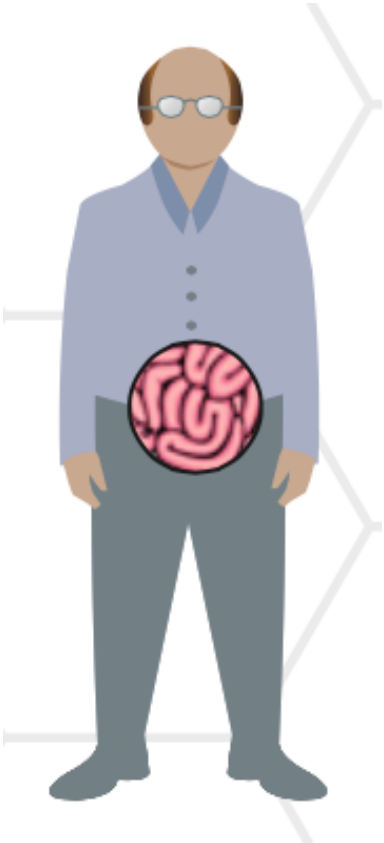
- Detectar e responder rapidamente para reduzir a MDR em gonorréia;

- Identificar intervenções críticas contra *M. tuberculosis* multi-resistentes, expandir avaliação de imigrantes e refugiados;

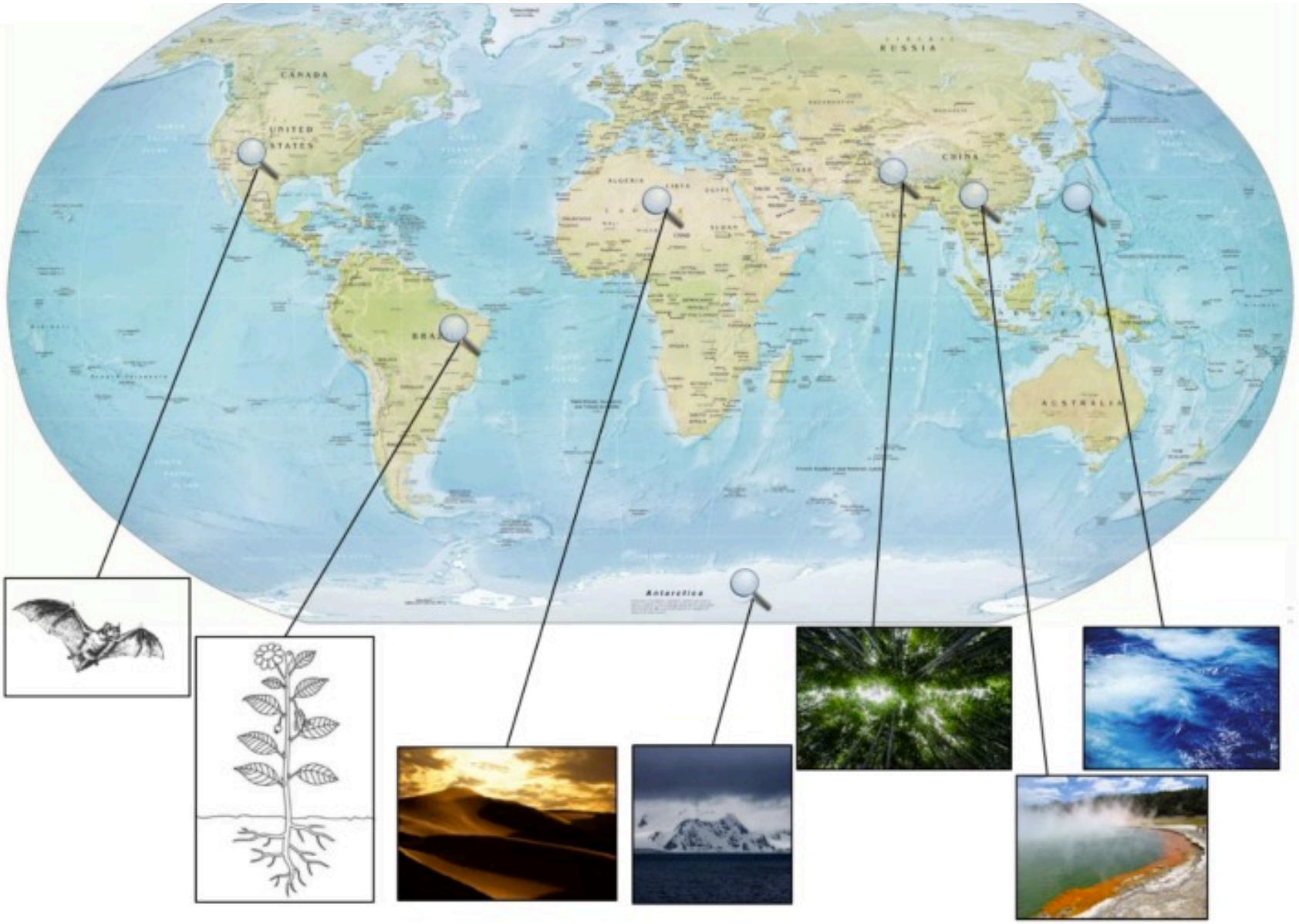


Plano de iniciativas para vencer a resistência

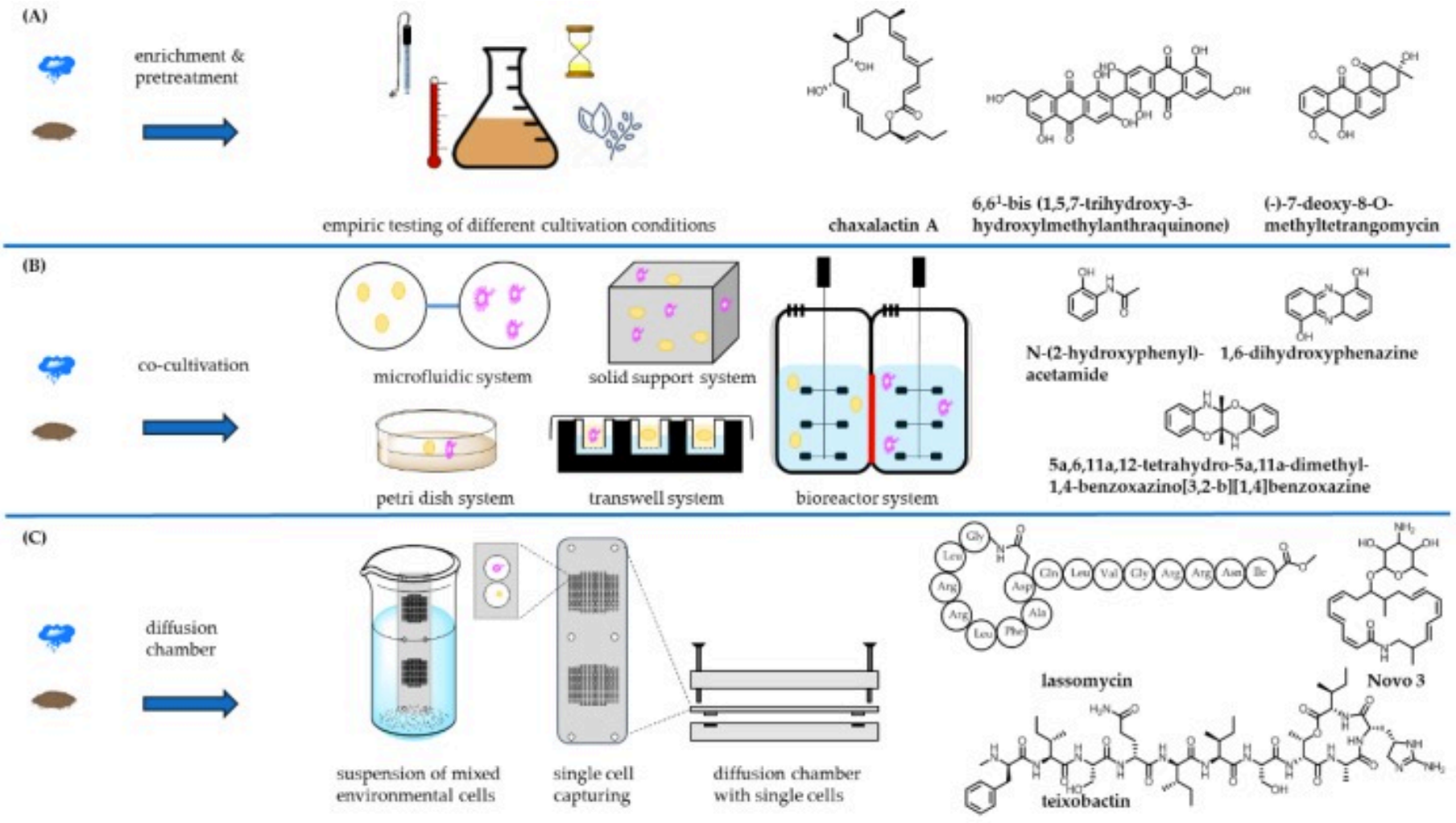
Avaliar impacto dos antibióticos no microbioma e avaliar como usar os microrganismos para controle



Underexploited habitats of actinobacteria attracted more attention for microbial natural product discovery. Currently, oceans, deserts, mountains and Antarctica ranges together with hot springs and endophytes and symbionts are focuses of the search for new bioactive compounds.



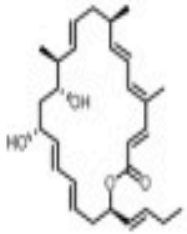
Isolation strategies. (**A**) Soil sample or marine sample undergoes enrichment and/or pretreatment to increase the chance to isolate new species and/or reduce undesirable background from previously isolated strains. Methods for fermentation varies in incubation time, media composition, additives, pH and temperature to enable growth of desirable strains.. (**B**) Sample is co-cultivated with other microorganisms to promote culturable isolates or to stimulate the secondary metabolism. (**C**) Sample is used to create a suspension of mixed environmental cells. The isolation chip (iChip) plate is immersed into this suspension to capture (on average) a single cell.



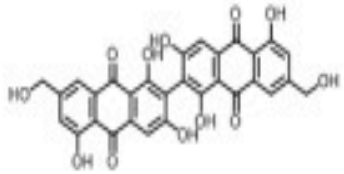
(A)

enrichment & pretreatment

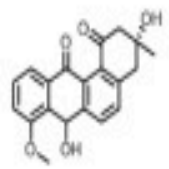
empiric testing of different cultivation conditions



chaxalactin A



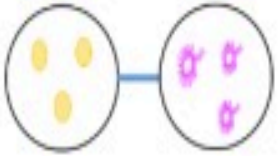
6,6[1]-bis (1,5,7-trihydroxy-3-hydroxylmethylanthraquinone)



(-)-7-deoxy-8-O-methyltetrangomycin

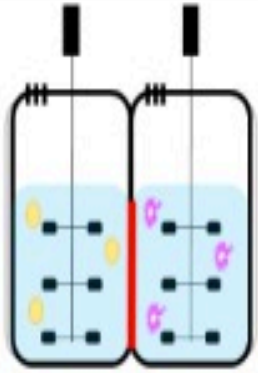
(B)

co-cultivation



microfluidic system

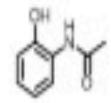
solid support system



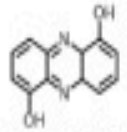
bioreactor system

petri dish system

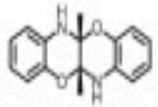
transwell system



N-(2-hydroxyphenyl)-acetamide



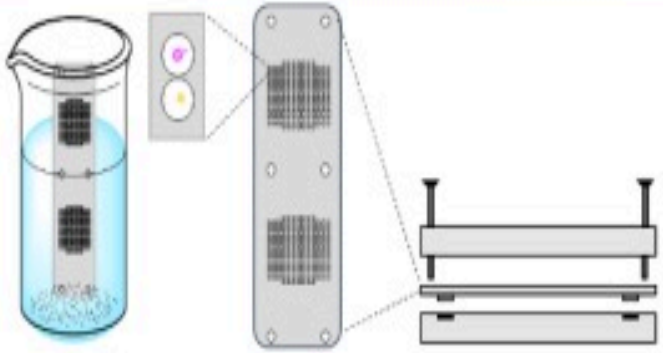
1,6-dihydroxyphenazine



5a,6,11a,12-tetrahydro-5a,11a-dimethyl-1,4-benzoxazino[3,2-b][1,4]benzoxazine

(C)

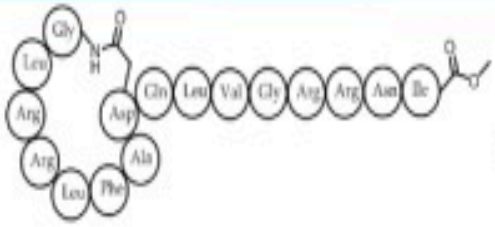
diffusion chamber



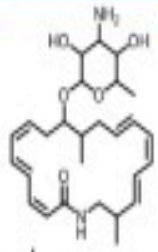
suspension of mixed environmental cells

single cell capturing

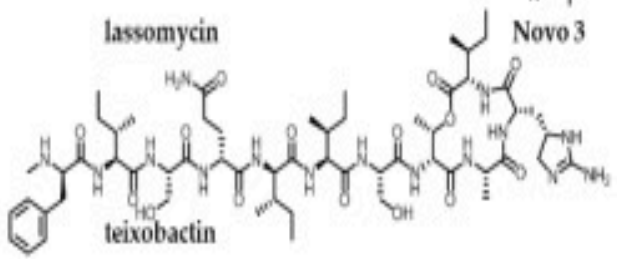
diffusion chamber with single cells



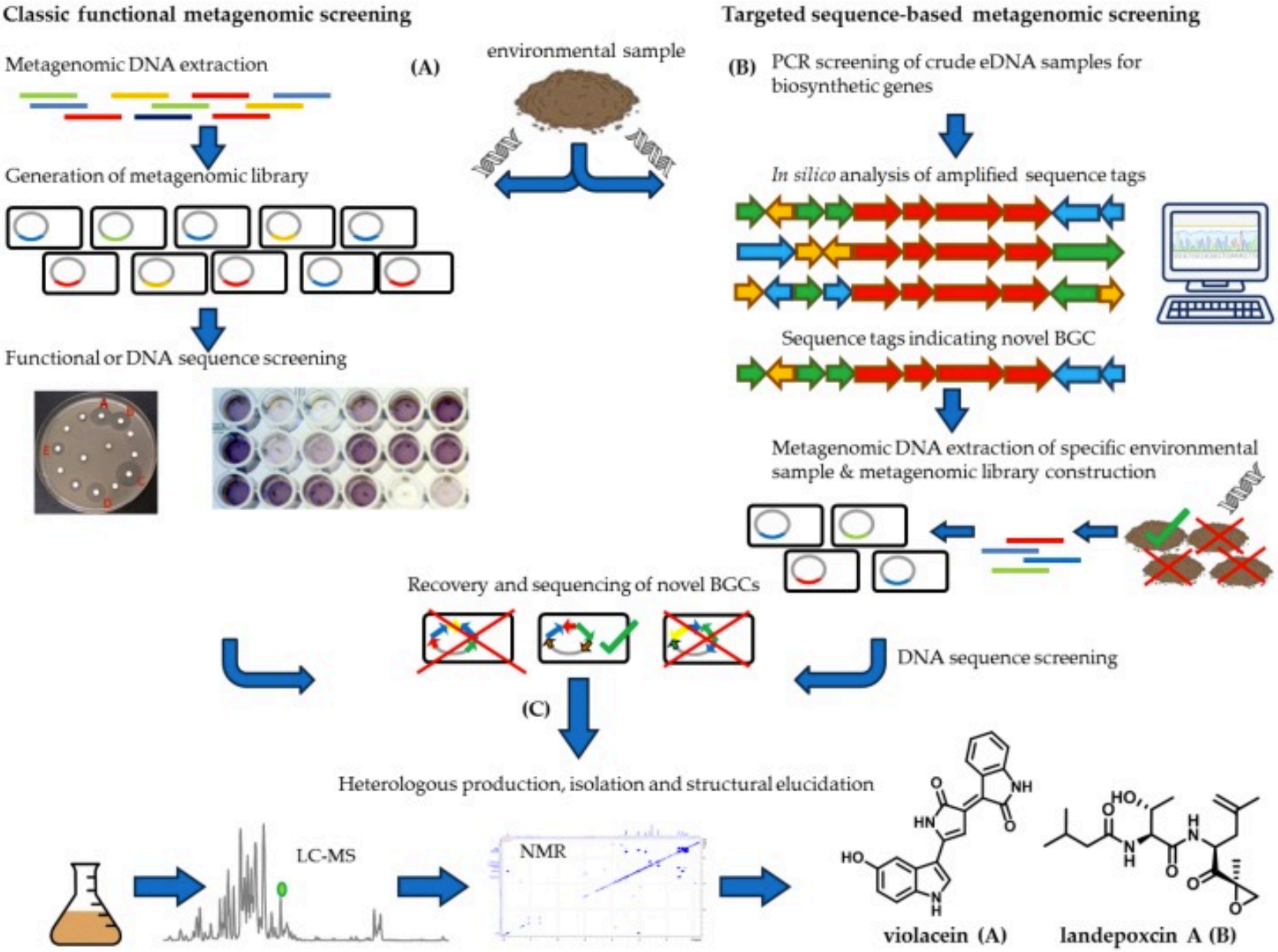
lassomycin

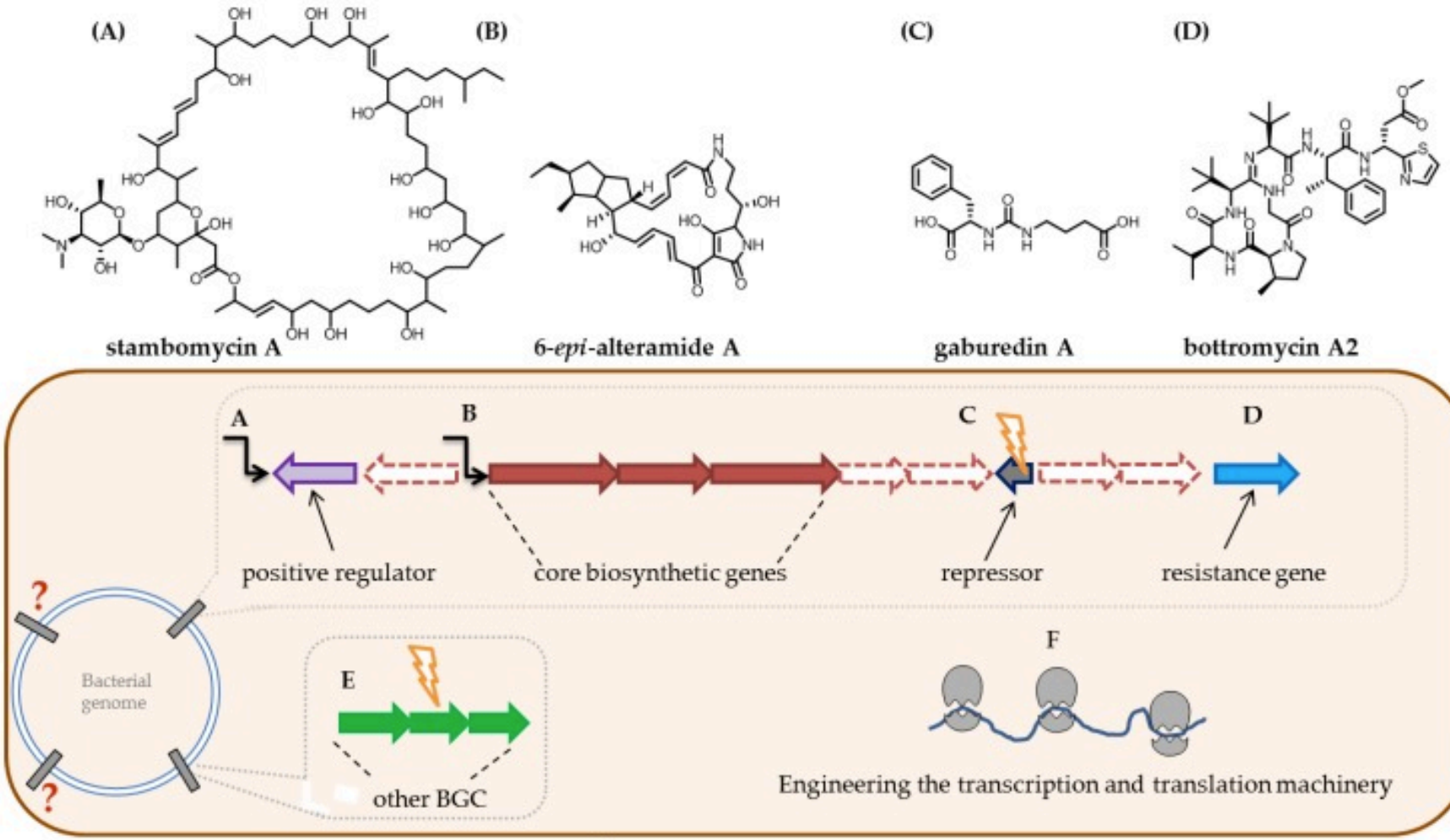


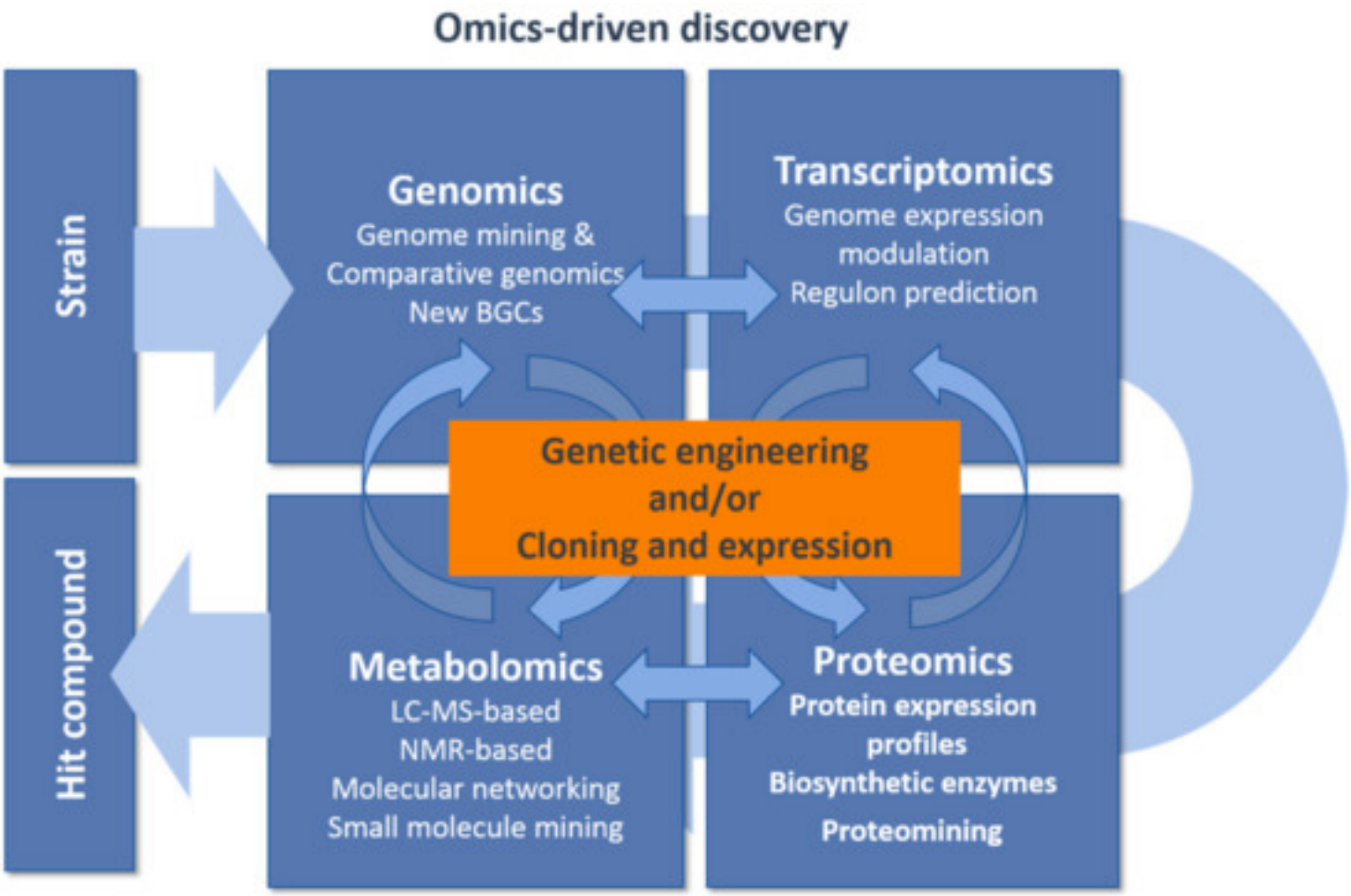
Novo 3



teixobactin


Classic functional metagenomic screening
Targeted sequence-based metagenomic screening
Metagenomic DNA extraction
(A)
environmental sample
(B) PCR screening of crude eDNA samples for biosynthetic genes
Generation of metagenomic library
In silico analysis of amplified sequence tags
Functional or DNA sequence screening
Sequence tags indicating novel BGC
Metagenomic DNA extraction of specific environmental sample & metagenomic library construction
Recovery and sequencing of novel BGCs
DNA sequence screening
(C)
Heterologous production, isolation and structural elucidation
LC-MS
NMR
violacein (A)
landepoxcin A (B)


(A)
(B)
(C)
(D)
stambomycin A
6-epi-alteramide A
gaburedin A
bottromycin A2
A
B
C
D
positive regulator
core biosynthetic genes
repressor
resistance gene
Bacterial genome
E
other BGC
F
Engineering the transcription and translation machinery
(E)
(F)
amexanthomycin A
piperidamycin A


Omics-driven discovery
Strain
Hit compound
Genomics
Genome mining &
Comparative genomics
New BGCs
Transcriptomics
Genome expression
modulation
Regulon prediction
Genetic engineering
and/or
Cloning and expression
Metabolomics
LC-MS-based
NMR-based
Molecular networking
Small molecule mining
Proteomics
Protein expression
profiles
Biosynthetic enzymes
Proteomining