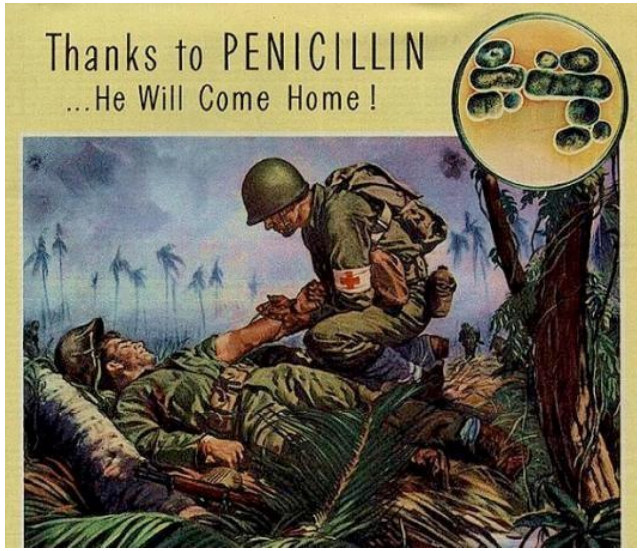
Laboratory of Physical Chemistry and Microbiology for the Environment

Antibiotic resistance: an issue of emerging concern

Christophe MERLIN



Advertisement for penicillin production from Life magazine, August 14, 1944.

Les antibiotiques

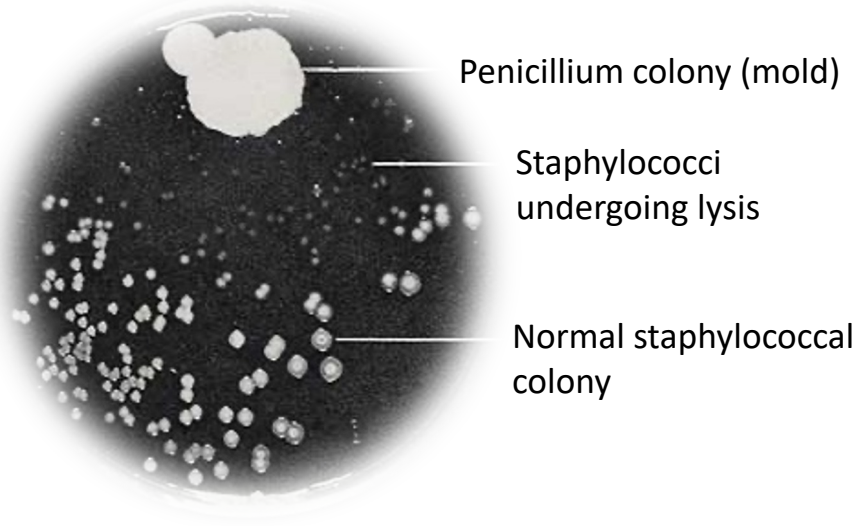
From antibiotics discovery to antibiotic resistance

A little bit of history…

Alexander Fleming

1928 Discovery of penicillin

1945 Nobel price (with H. Florey & E.B. Chain)



"dissolution of staphyloccocal colonies in the neighbourhood of a penicillium colony"

A short history of antibiotic discovery

Louis Pasteur

1857: *Report on the Lactic Acid Fermentation*

The modern Era of microbiology

Relationship between microorganisms and specific activities or diseases.

=> identification of etiological agents (pathogens)

=> fight against morbid entities (vaccines, antibiotics)

Act I - Identifying antagonist effects

Jean Paul Vuillemin

1889: Concept of antibiosis and symbiosis between organisms

Ernest Duchesne

1897: vital competition among microorganisms: antagonism between molds and microbes

Georges Papacostas and **Jean Gaté**

1927: Microbial associations: their therapeutic applications

Alexander Flemming

1928: antagonist effect of molds at the origin of **Penicillin** discovery

1945: Nobel Prize (with H. Florey & E.B. Chain)

The beginning of the antibiotic Era

"think globally, act locally"

Act II – Isolating active molecules

René Dubos

1927: PhD in soil microbiology (Waksman lab)
1930: identification of **S III enzyme**, effective against *Pneumococcus* in mice (Avery lab)
1939: identification of **gramicidin** (1st antibiotic released into the market)

=> **Methodology**: Soil bacteria trapping and active substance identification

Gerhard Domagk

1935: identification and use of antagonist substances: sulfonamides (prontosil dye)
1939: Nobel Prize (refused)

Howard Florey and Ernst Chain

1940: identification and production of penicillin
1945: Nobel Prize (with A. Fleming)

Selman Waksman

1930-1950s: discovery of 22 antibiotics from actinomycetes
1930s: inventor of the word "antibiotics"
1952: Nobel Prize (Streptomycin: background, isolation, properties, and utilization)

R. Dubos et Gramicidine

STUDIES ON A BACTERICIDAL AGENT EXTRACTED FROM A SOIL BACILLUS

I. Preparation of the Agent. Its Activity in Vitro

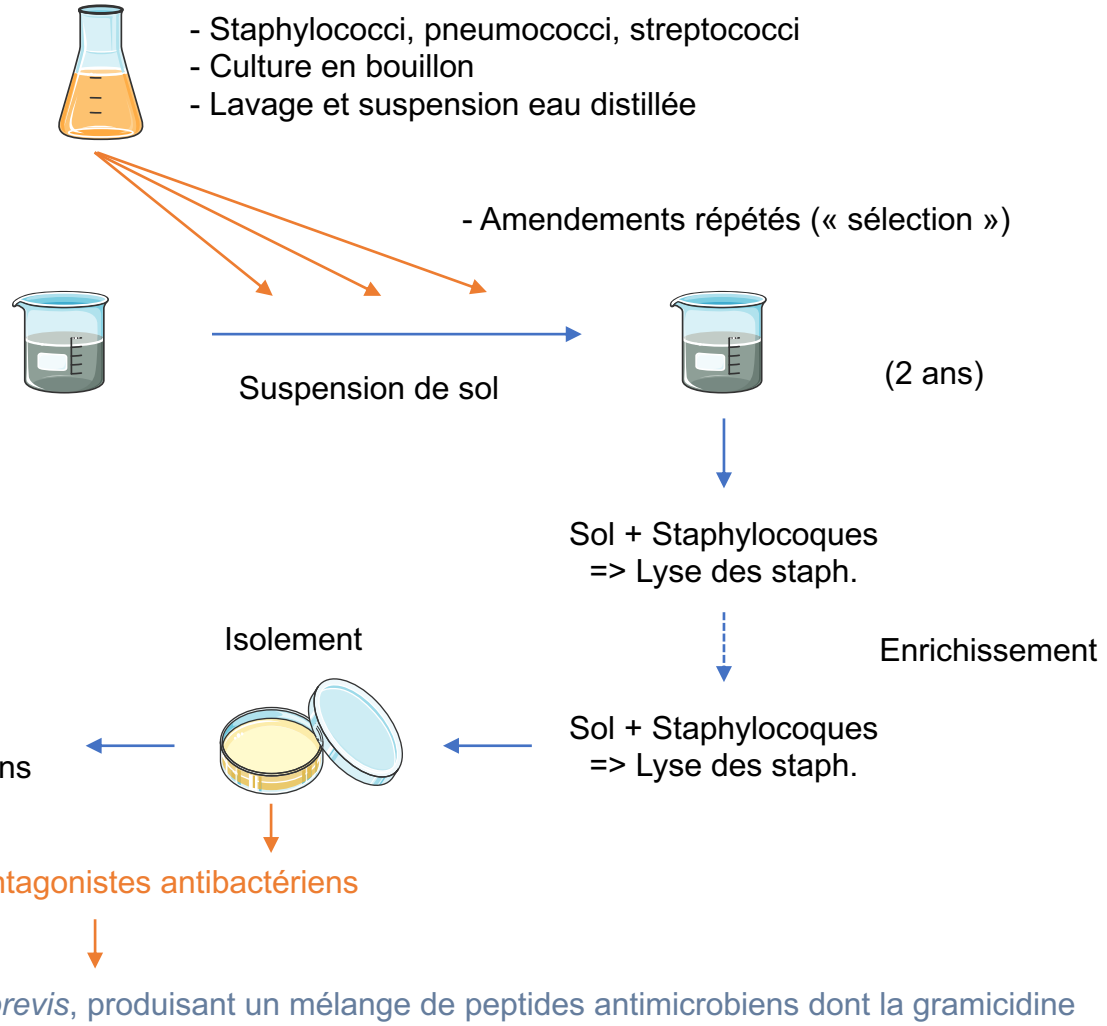
By RENÉ J. DUBOS, Ph.D.

(*From the Hospital of The Rockefeller Institute for Medical Research*)

(Received for publication, April 17, 1939)

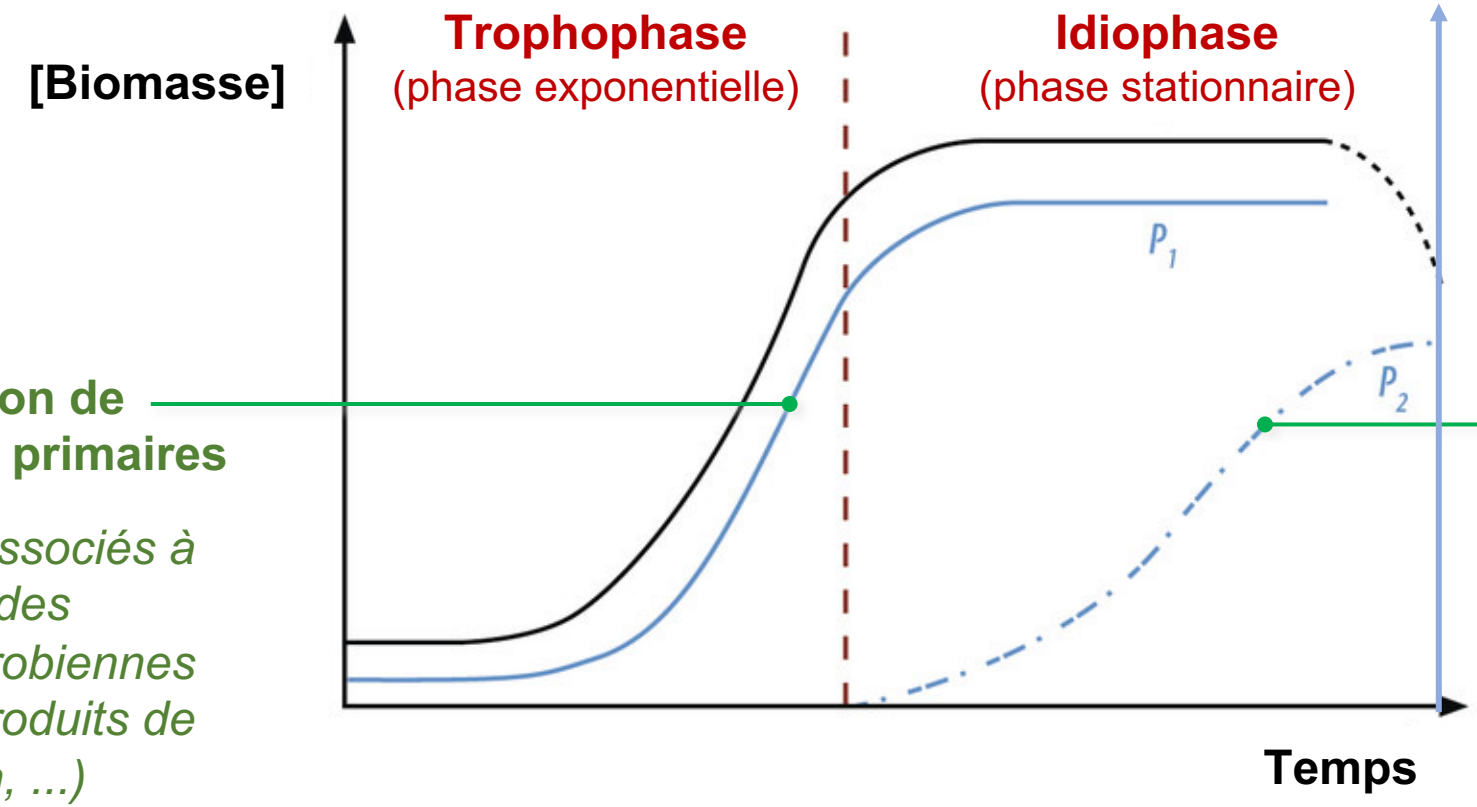
Microorganisms perform a vast number of biochemical reactions, many of which are not known to occur in the animal and plant kingdoms (1). On the basis of present knowledge it is conceivable that one may find in nature microbial species endowed with catalysts capable of activating almost any type of biochemical reaction. During the past few years, this point of view has found its application in the isolation of soil microorganisms which selectively attack certain substances of interest to the biochemist (2) and to the immunologist (3–8). It may be recalled in particular that soluble polysaccharides, extracted from several bacterial pathogens, have been found to be decomposed by certain microbial species, although the same substances are resistant to the action of all known enzymes of animal and plant origin.

It appeared possible that there also exist in nature microorganisms capable of attacking not only isolated soluble components of other bacterial cells, but also the intact living cells themselves. Actually we have isolated from soil a spore-bearing bacillus which attacks and lyzes the living cells of several species of Gram-positive microorganisms. The present paper describes the isolation of this new soil bacillus, and the preparation, properties, and activity of the soluble agent by means of which it attacks and lyzes the living cells of the susceptible, Gram-positive species.



Dubos, 1939

Croissance microbienne et production de métabolites



Formation de métabolites primaires

composés associés à la synthèse des cellules microbiennes (A.A., Nt., produits de fermentation, ...)

Formation de métabolites secondaires

*composés sans relation directe avec la croissance (**antibiotiques**, toxines, ...*

The interest of secondary metabolites

22,000 bioactive compounds (2002)

(1) Enzyme inhibitors which are **cholesterol-lowering agents**: statins [lovastatine, pravastatine (pravacol), simvastatine (Zocor), atorvastatine (Lipitor)].

(2) Immunosuppressants for organ transplantation: cyclosporine, sirolimus, tacrolimus, mycophenolic acid.

(3) antiparasitic agents: antihelmintics (avermectins), coccidiostats and ruminant growth promoters (monensin,narasin, lasalocid, salinomycin)

(4) bio-herbicides (bialaphos)

(5) plant growth regulators (gibberellins)

(6) bio-pesticides (kasugamycin, polyoxins)

(7) bio-insecticides (spinosins and nikkomycin)

(8) Antitumor agents (doxorubicin, daunorubicin, mitomycin, bléomycin, etc.)

(9) Antibiotics (20,000 compounds discovered but not all are usable!)

Demain (2006) J. Ind Microbiol. Biotechnol. 33:486-495

métabolites secondaires et antibiotiques

Le pouvoir économique des microbes

en 2000 on estimait du marché annuel des métabolites secondaires

62.445 millions de $

Les antibiotiques

en 2002 on dénombrait 22.000 composés bioactifs découverts chez les microbes, incluant 20.000 antibiotiques!

Les limitations

(1) Toutes les molécules bioactives ne sont pas utilisables

(2) Conditions de culture et optimisation.

(3) 1% de bactéries cultivables.

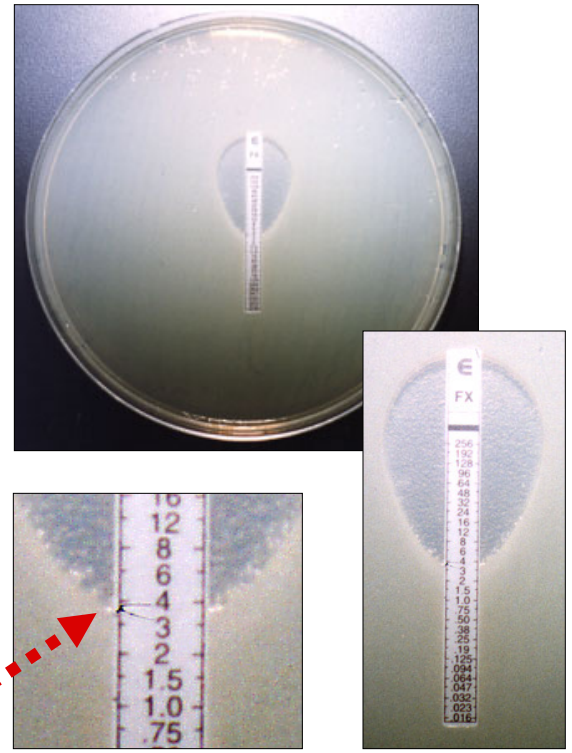
... les composés bioactifs naturels sont encore « sous-explorés »

Demain (2006) J. Ind Microbiol. Biotechnol. 33:486-495

Effects of antibiotics on bacteria

[Antibiotic]

No growth in presence of the Ab
Minimum Inihibitory Concentration (**MIC**)



(ETest)

No growth after Ab removal
Minimal Bactericidal Concentration (**MBC**)

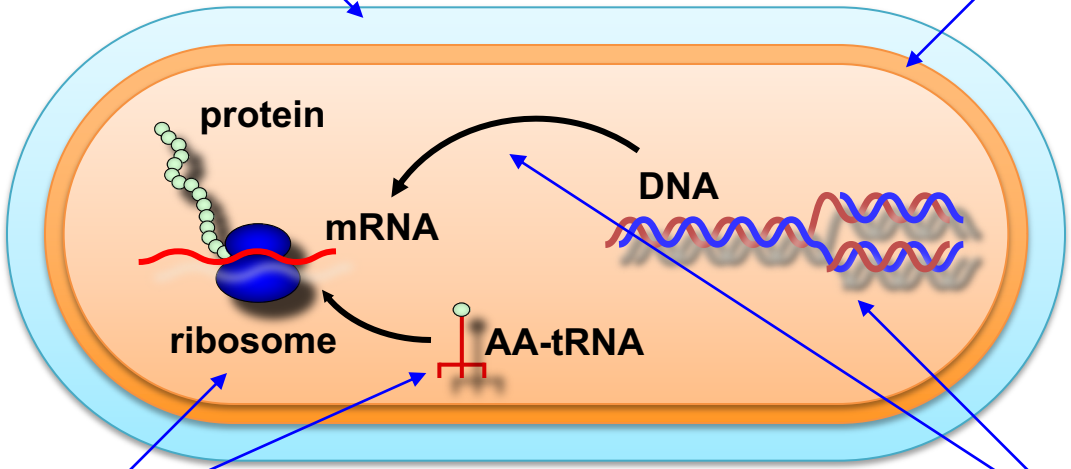
Antibiotics : modes of action and targets

1 Inhibition of the cell wall synthesis

- *Inhibition of cell wall precursors synthesis*
- *Inhibition of precursor transport*
- *Inhibition of cell wall assembly*

2 Alteration of the cytoplasmic membrane integrity

Alteration of membrane permeability



3 Inhibition of protein synthesis

- *Inhibition of the translation (ribosome, elongation factor EF-G)*
- *Inhibition of amino acyl-tRNA synthetase*

4 Inhibition of DNA metabolism

- *Inhibition of transcription (RNA polymerase),*
- *Inhibition of nucleic acids precursor synthesis,*
- *Inhibition of DNA replication (gyrase, topoisomerase IV)*

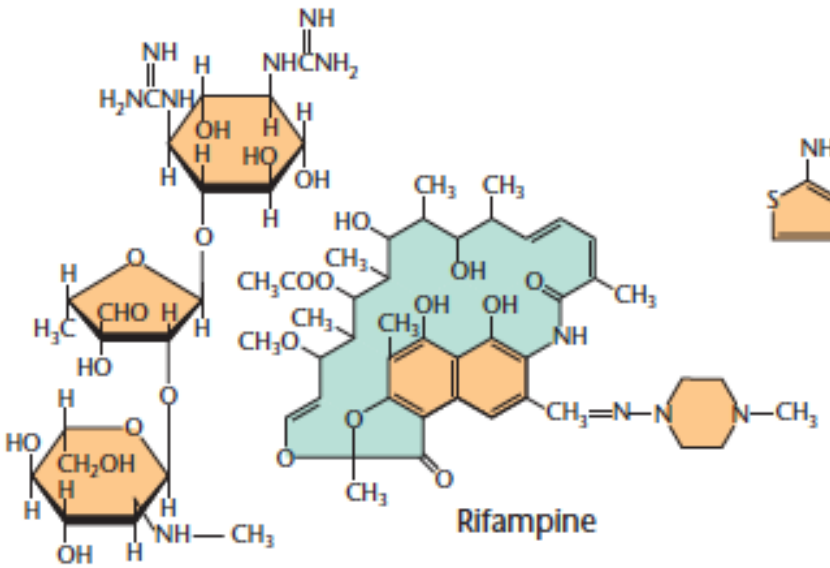
5 Others

vitamin analogues (competitive inhibition)

Exemples de structures d'antimicrobiens

Les agents chimiothérapeutiques antibactériens sont classés selon **leur structure chimique**, leur **mode d'action** ou leur **spectre d'activité**.

Structure représentative

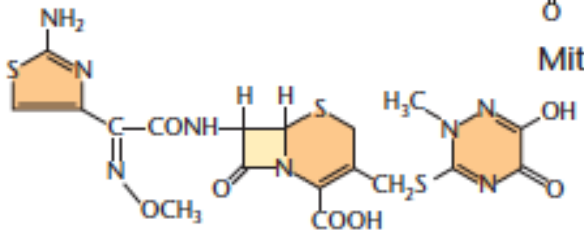


Streptomycine

(I)

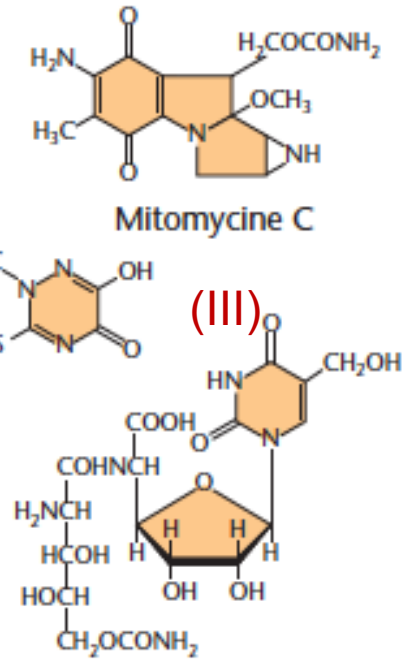
Rifampine

(II)



Ceftriaxone

(IV)

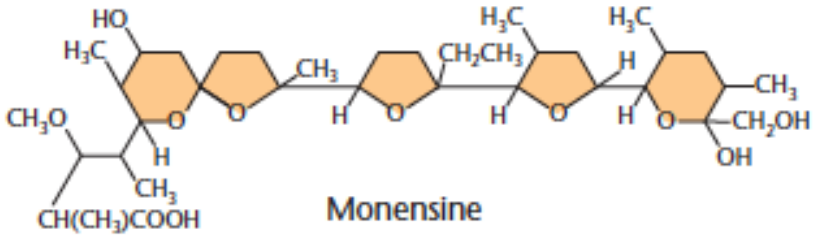


Polyoxine B

(V)

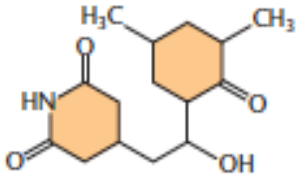
Mitomycine C

(III)

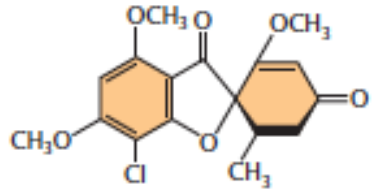


Monensine

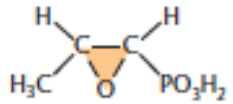
(VI)



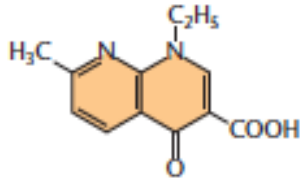
Cycloheximide (VII)



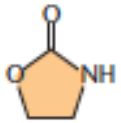
Griséofulvine (VIII)



Fosfomycine (IX)



Acide nalidixique (X)



2-Oxazolidinone (XI)

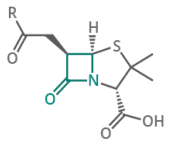
extrait de « Brock – Biologie des microorganismes » (11e édition)

DIFFERENT CLASSES OF ANTIBIOTICS - AN OVERVIEW

Key: COMMONLY ACT AS **BACTERIOSTATIC AGENTS**, RESTRICTING GROWTH & REPRODUCTION — COMMONLY ACT AS **BACTERICIDAL AGENTS**, CAUSING BACTERIAL CELL DEATH

ß-LACTAMS

MOST WIDELY USED ANTIBIOTICS IN THE NHS



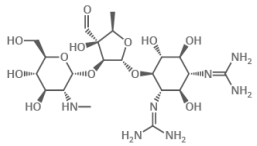
All contain a beta-lactam ring

EXAMPLES
Penicillins (shown) such as amoxicillin and flucloxacillin; *Cephalosporins* such as cefalexin.

MODE OF ACTION
Inhibit bacteria cell wall biosynthesis.

AMINOGLYCOSIDES

FAMILY OF OVER 20 ANTIBIOTICS



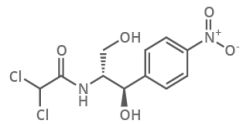
All contain aminosugar substructures

EXAMPLES
Streptomycin (shown), neomycin, kanamycin, paromomycin.

MODE OF ACTION
Inhibit the synthesis of proteins by bacteria, leading to cell death.

CHLORAMPHENICOL

COMMONLY USED IN LOW INCOME COUNTRIES



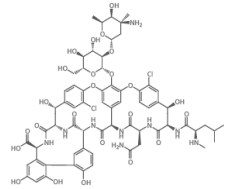
Distinct individual compound

MODE OF ACTION
Inhibit synthesis of proteins, preventing growth.

No longer a first line drug in any developed nation due to increased resistance and worries about safety.

GLYCOPEPTIDES

COMMON 'DRUGS OF LAST RESORT'



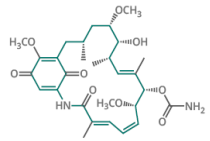
Consist of carbohydrate linked to a peptide formed of amino acids

EXAMPLES
Vancomycin (shown), teicoplanin.

MODE OF ACTION
Inhibit bacteria cell wall biosynthesis.

ANSAMYCINS

CAN ALSO DEMONSTRATE ANTIVIRAL ACTIVITY



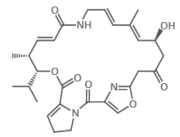
All contain an aromatic ring bridged by an aliphatic chain.

EXAMPLES
Geldanamycin (shown), rifamycin, naphthomycin.

MODE OF ACTION
Inhibit the synthesis of RNA by bacteria, leading to cell death.

STREPTOGRAMINS

TWO GROUPS OF ANTIBIOTICS THAT ACT SYNERGISTICALLY



Combination of two structurally differing compounds, from groups denoted A & B

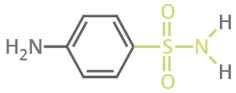
EXAMPLES
Pristinamycin IIA (shown), Pristinamycin IA.

MODE OF ACTION
Inhibit the synthesis of proteins by bacteria, leading to cell death.

DISCOVERY — 1930 — 1940 — 1950 — 1960 — 1970 — 1980

SULFONAMIDES

FIRST COMMERCIAL ANTIBIOTICS WERE SULFONAMIDES



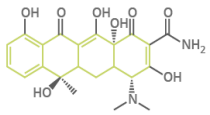
All contain the sulfonamide group

EXAMPLES
Prontosil, sulfanilamide (shown), sulfadiazine, sulfisoxazole.

MODE OF ACTION
Do not kill bacteria but prevent their growth and multiplication. Cause allergic reactions in some patients.

TETRACYCLINES

BECOMING LESS POPULAR DUE TO DEVELOPMENT OF RESISTANCE



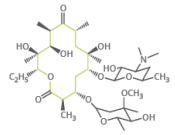
All contain 4 adjacent cyclic hydrocarbon rings

EXAMPLES
Tetracycline (shown), doxycycline, limecycline, oxytetracycline.

MODE OF ACTION
Inhibit synthesis of proteins by bacteria, preventing growth.

MACROLIDES

SECOND MOST PRESCRIBED ANTIBIOTICS IN THE NHS



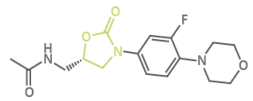
All contain a 14-, 15-, or 16-membered macrolide ring

EXAMPLES
Erythromycin (shown), clarithromycin, azithromycin.

MODE OF ACTION
Inhibit protein synthesis by bacteria, occasionally leading to cell death.

OXAZOLIDINONES

POTENT ANTIBIOTICS COMMONLY USED AS 'DRUGS OF LAST RESORT'



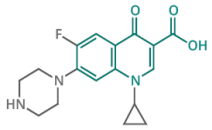
All contain 2-oxazolidone somewhere in their structure

EXAMPLES
Linezolid (shown), posizolid, tedizolid, cycloserine.

MODE OF ACTION
Inhibit synthesis of proteins by bacteria, preventing growth.

QUINOLONES

RESISTANCE EVOLVES RAPIDLY



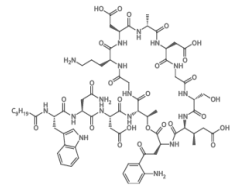
All contain fused aromatic rings with a carboxylic acid group attached

EXAMPLES
Ciprofloxacin (shown), levofloxacin, trovafloxacin.

MODE OF ACTION
Interfere with bacteria DNA replication and transcription.

LIPOPEPTIDES

INSTANCES OF RESISTANCE RARE



All contain a lipid bonded to a peptide

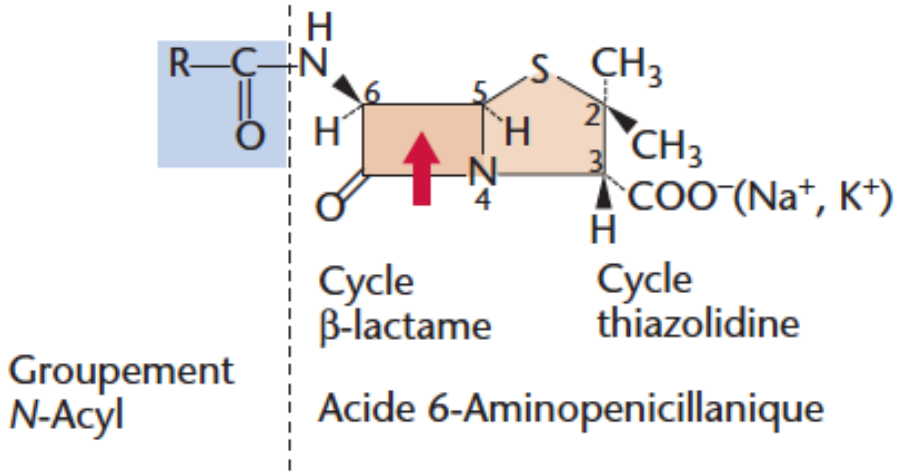
EXAMPLES
Daptomycin (shown), surfactin.

MODE OF ACTION
Disrupt multiple cell membrane functions, leading to cell death.

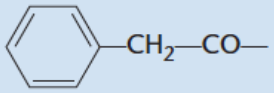
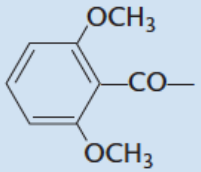
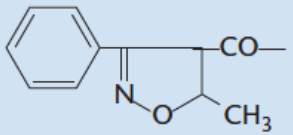
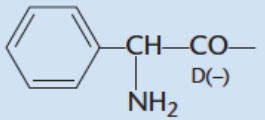
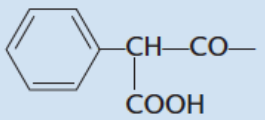
Variations sur un même thème

Des modifications de structure modulent le mode d'action et le spectre d'activité des antimicrobiens.

Exemple : structure des pénicillines



Structurellement les pénicillines se composent de 3 parties et se distinguent par leur groupement N-Acyl.

Appellation	Groupe *N*-Acyl
Pénicilline naturelle	
Benzylpénicilline (pénicilline G) Activité contre les bactéries Gram positif, sensible aux β-lactamases	
Pénicillines semi-synthétiques	
Méthicilline Acide stable, résistant aux β-lactamases	
Oxacilline Acide stable, résistant aux β-lactamases	
Ampicilline Large spectre d'activité (spécialement contre les bactéries Gram négatif) Acide stable, résistant aux β-lactamases	
Carbénicilline Large spectre d'activité (spécialement contre *Pseudomonas aeruginosa*) Acide stable mais inefficace par voie orale, sensible aux β-lactamases	

Pénicilline naturelle isolée par Fleming à partir de moisissure (*Penicillium chrysogenum)*

Beaucoup d'antibiotiques naturels ont été structurellement modifiés en laboratoire pour augmenter leur efficacité, formant le groupe des antibiotiques **semi-synthétiques.**

extrait de « Brock – Biologie des microorganismes » (11e édition)

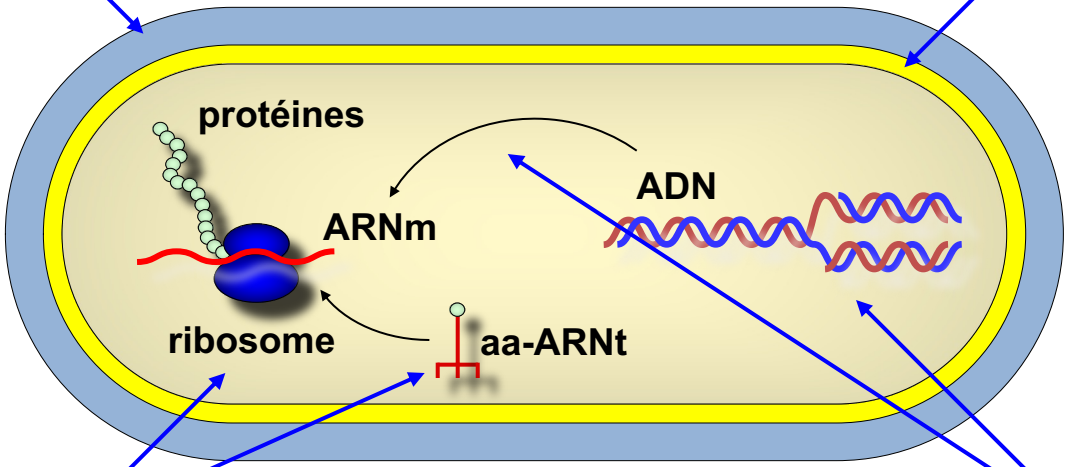
Modes d'action des agents thérapeutiques antibactériens

1 Inhibition de la synthèse de la paroi bactérienne

blocage synthèse, transfert ou assemblage des précurseurs du peptidoglycane

2 Altération de l'intégrité de la membrane cytoplasmique

désorganisation de la bicouche (surfactant)



(bactérie)

3 Inhibition de la synthèse protéique

blocage de la traduction (ribosome, facteur d'élongation EF-G, AA-tRNA synthase)

4 Action sur le métabolisme de l'ADN

blocage transcription (ARN polymérase), réplication (gyrase, topoisomérase IV), synthèse des acides nucléiques

5 Autre

analogues vitamine

Exemples d'agents antimicrobiens et leurs cibles

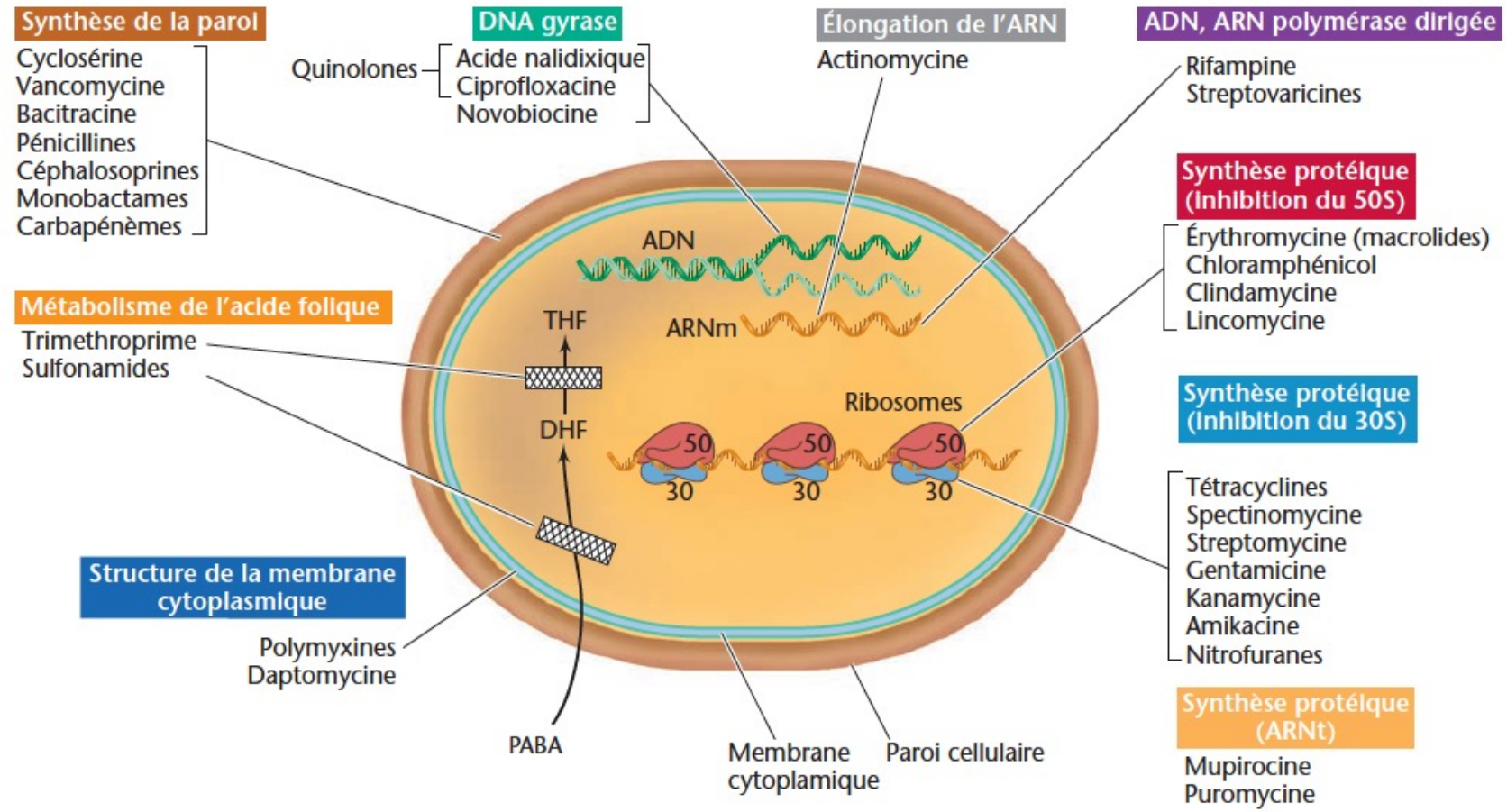


FIGURE 20.14 **Mode d'action des agents majeurs chimiothérapeutiques antimicrobiens.** THF, Tétrahydrofolate ; DHF, Dihydrofolate ; ARN messager (ARNm), ARN de transfert (ARNt).

extrait de « Brock – Biologie des microorganismes » (11e édition)

Exemples d'agents antimicrobiens et leurs spectres d'action

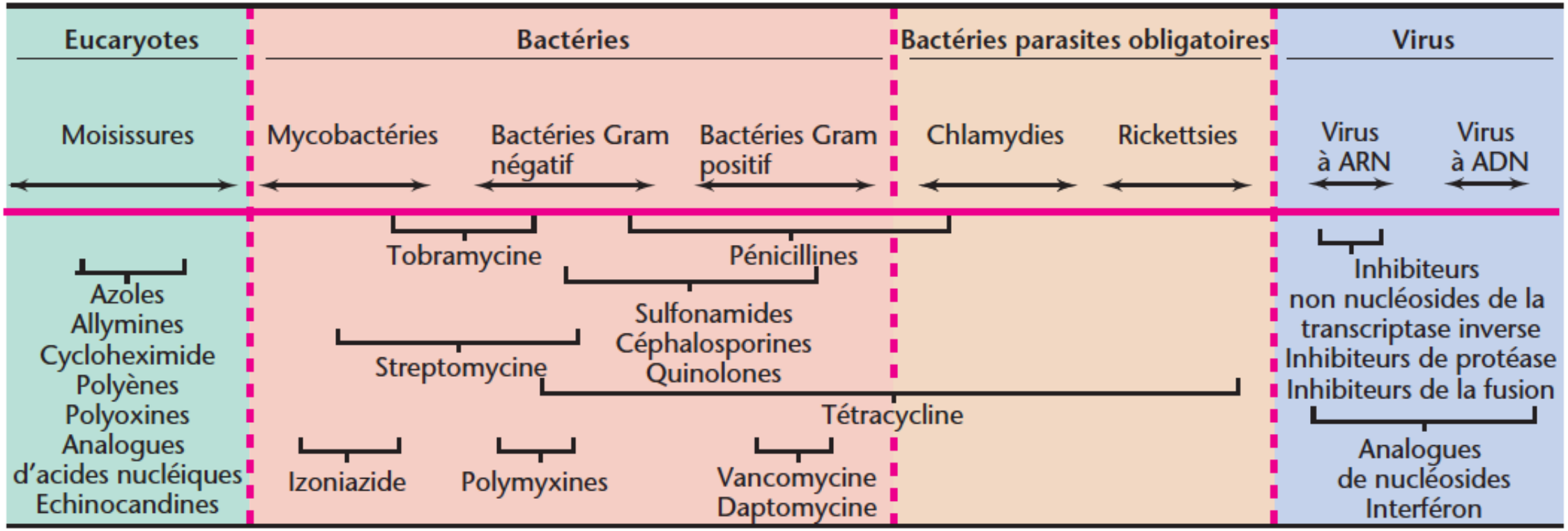


FIGURE 20.15 Spectre d'action des agents antimicrobiens. Chaque agent chimiothérapeutique antimicrobien affecte un groupe limité de micro-organismes.

=> importance de l'identification microbienne pour le choix thérapeutique

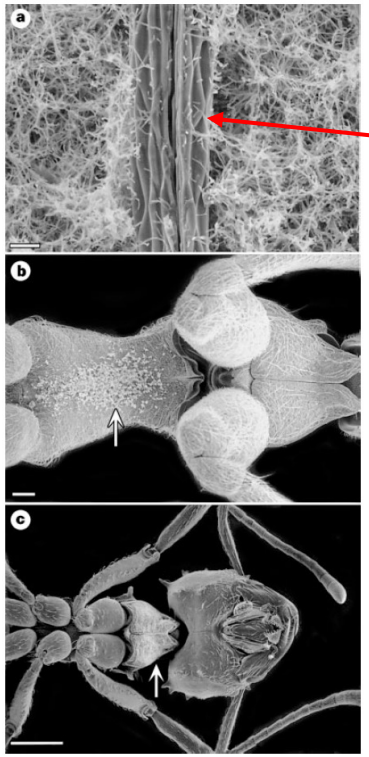
extrait de « Brock – Biologie des microorganismes » (11e édition)

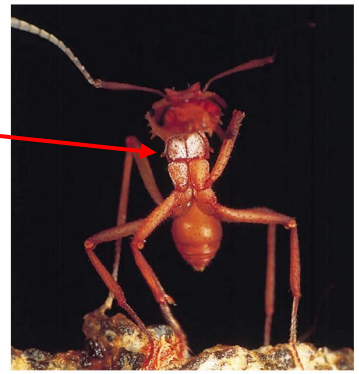
What are antibiotics for... territorial warfare?

Leafcutter ants : an example of biocontrol involving antibiotic producing bacteria

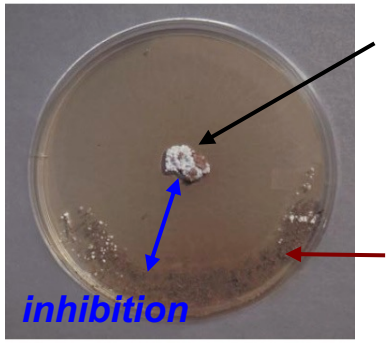
Attin ant (or ant fungus) farmer: underground culture of fungus

Currie et al. (1999) Nature 398:701-705





cuticle covered by actinomycetes



Actinomycetes

layer of *Escovopsis* (fungal parasite)

But... *"The existence of microbes that have the capacity to produce antibiotics in artificial culture cannot be interpreted as signifying that such phenomena are important in controlling microbial populations in nature... Unless one accepts the argument that laboratory environments are natural, one is forced to conclude that antibiotics play no part in modifying or influencing living processes that normally occur in nature."* (S Waksman, 1961)

Antimicrobial drugs... *in summary*

Antimicrobial drugs.

- Two broad categories:
  - synthetic antimicrobial drugs
  - natural antimicrobial drugs (= antibiotics)
- kill or inhibit growth of microorganisms (in the host)
- selective toxicity (against pathogens)
- classified based on:
  - their molecular structure
  - their mechanism of action
  - their spectrum of antimicrobial activity

Remarks.

- Role of antibiotics in nature:
  - produced by microbes to fight other microbes (“territorial warfare”)
  - messenger molecules (?)
- Resistances: at least present in natural drug producers

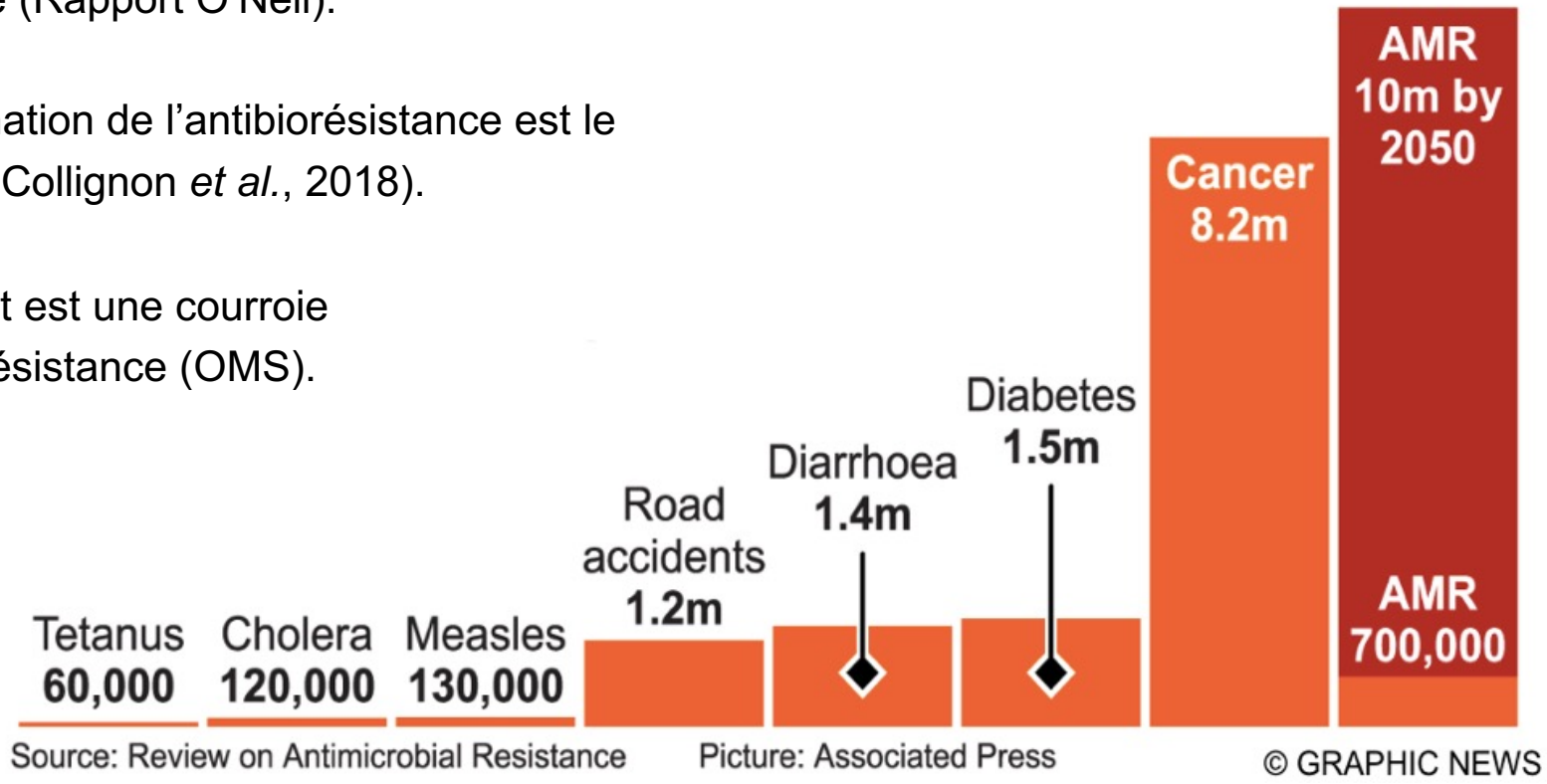
La résistance aux antibiotiques

Antibiorésistance, les enjeux pour la santé

Enjeu sociétal : à l'horizon 2050, la cause principale de mortalité sera liée à l'antibiorésistance (Rapport O'Neil).

Epidémiologie : la dissémination de l'antibiorésistance est le facteur contributif dominant (Collignon *et al.*, 2018).

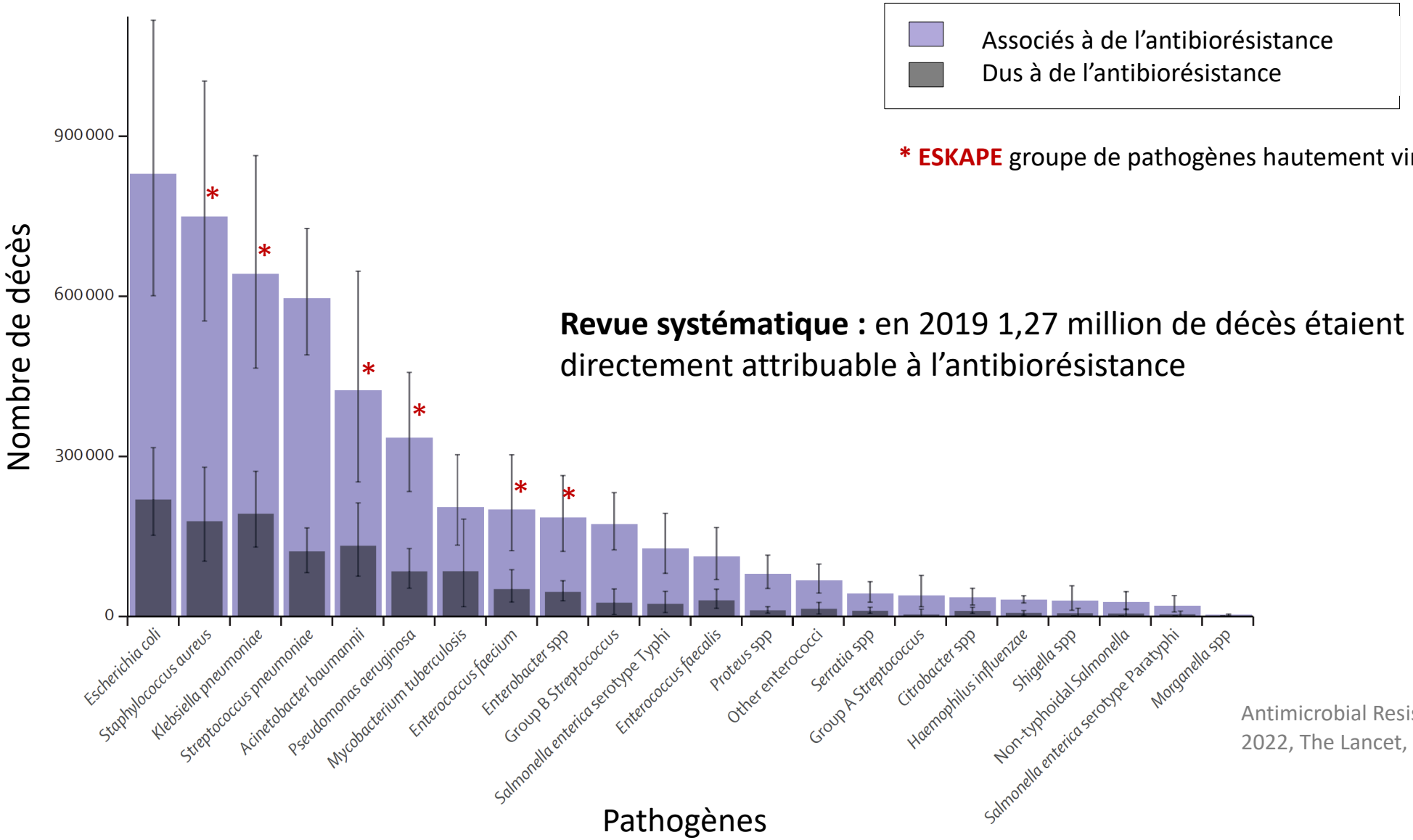
OneHealth : l'environnement est une courroie de transmission de l'antibiorésistance (OMS).



Attention, c'est une projection

D'après le rapport O'Neill (2016), une étude commandée à un économiste par le gouvernement britannique

Mortalité (mesurée) due à l'antibiorésistance en 2019

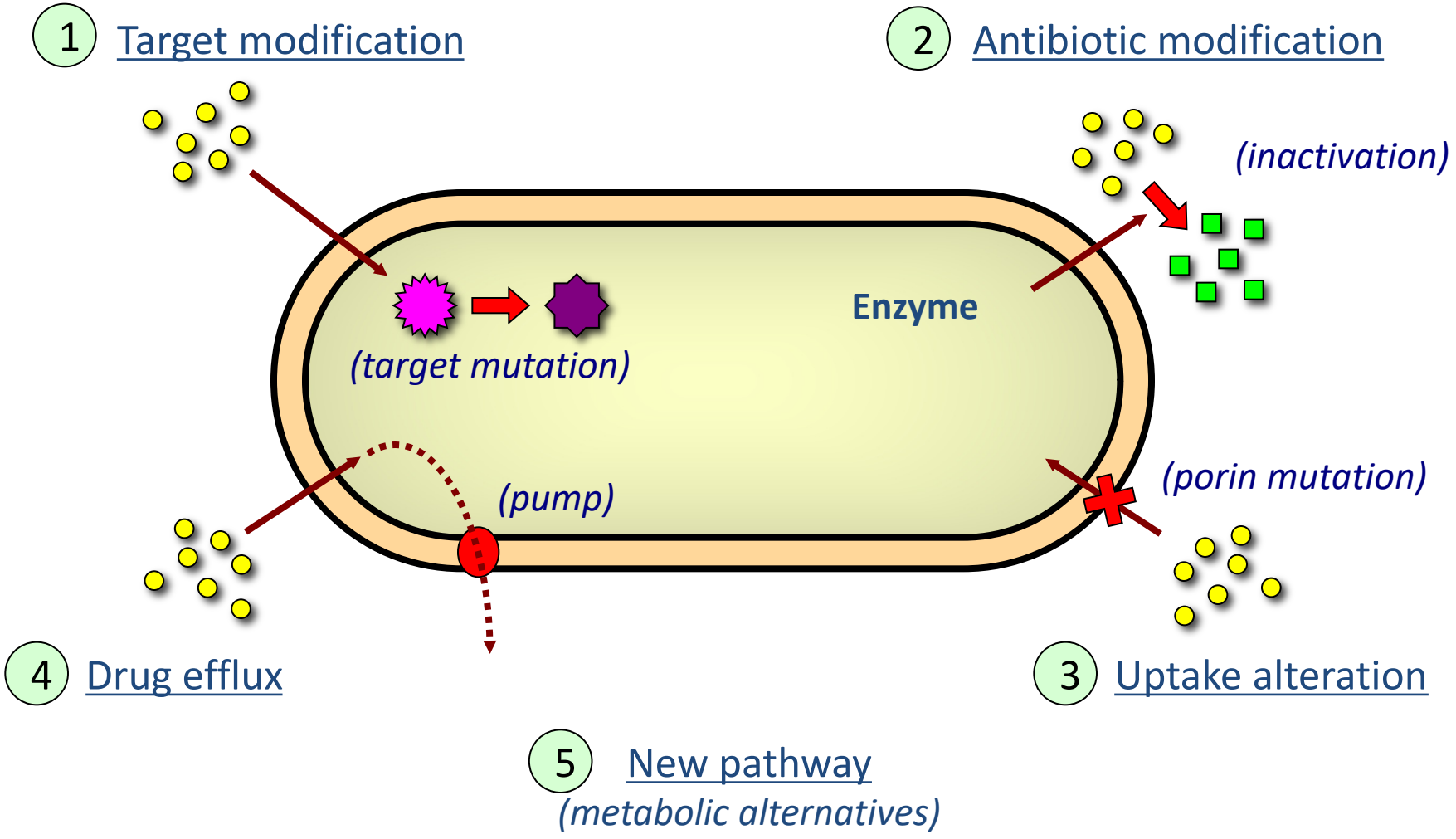


* **ESKAPE** groupe de pathogènes hautement virulents

Revue systématique : en 2019 1,27 million de décès étaient directement attribuable à l'antibiorésistance

Antimicrobial Resistance Collaborators, 2022, The Lancet, 399(10325):629-655

Major mechanisms involved in bacterial resistance to antimicrobials



Antimicrobial drug resistance

Absence of sensitivity to antimicrobials.

- Tolerance: naturally insensitive bacteria (no target, impermeability)
- Resistance: acquired ability to resist in a normally susceptible bacteria

Antimicrobial drug resistance.

- Reduce permeability
- Inactivation of antimicrobial
- Alteration of targets
- Development of resistant biochemical pathway
- Efflux

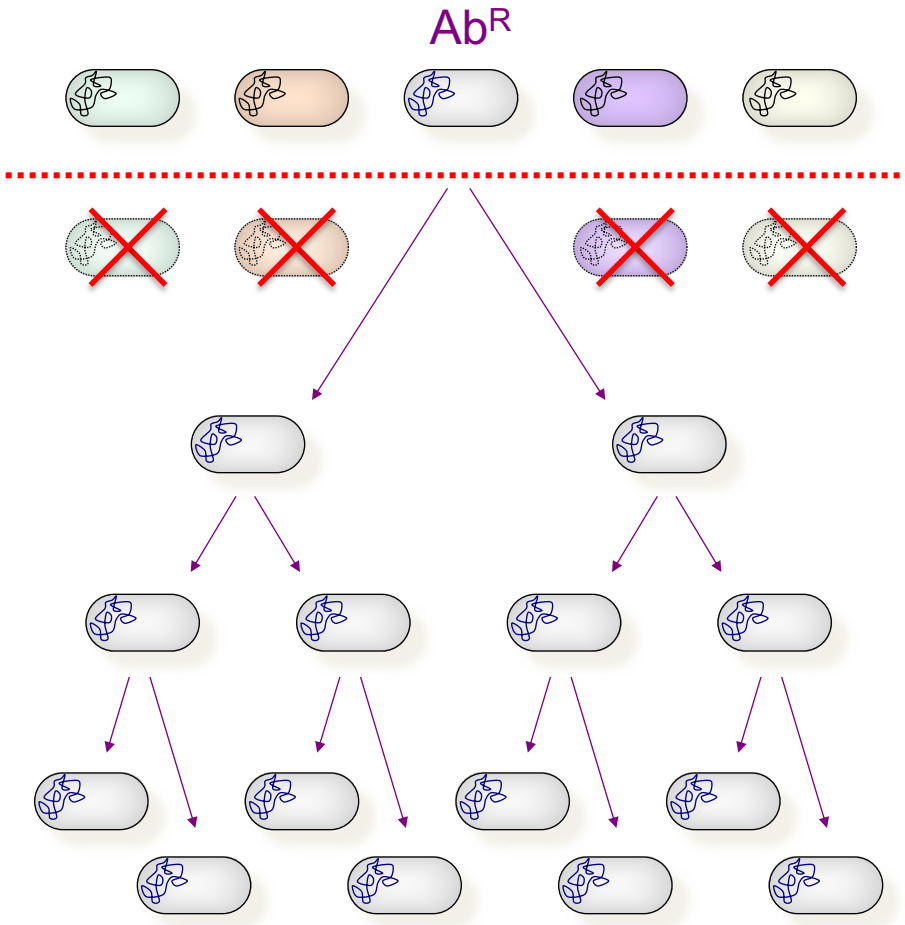
Acquisition of resistance (genetically acquired).

- Mutation: static
- Resistance genes: transferable

... or a combination

Consequences of a strong selective pressure

microbial community before Antibiotic pressure



Antibiotic pressure

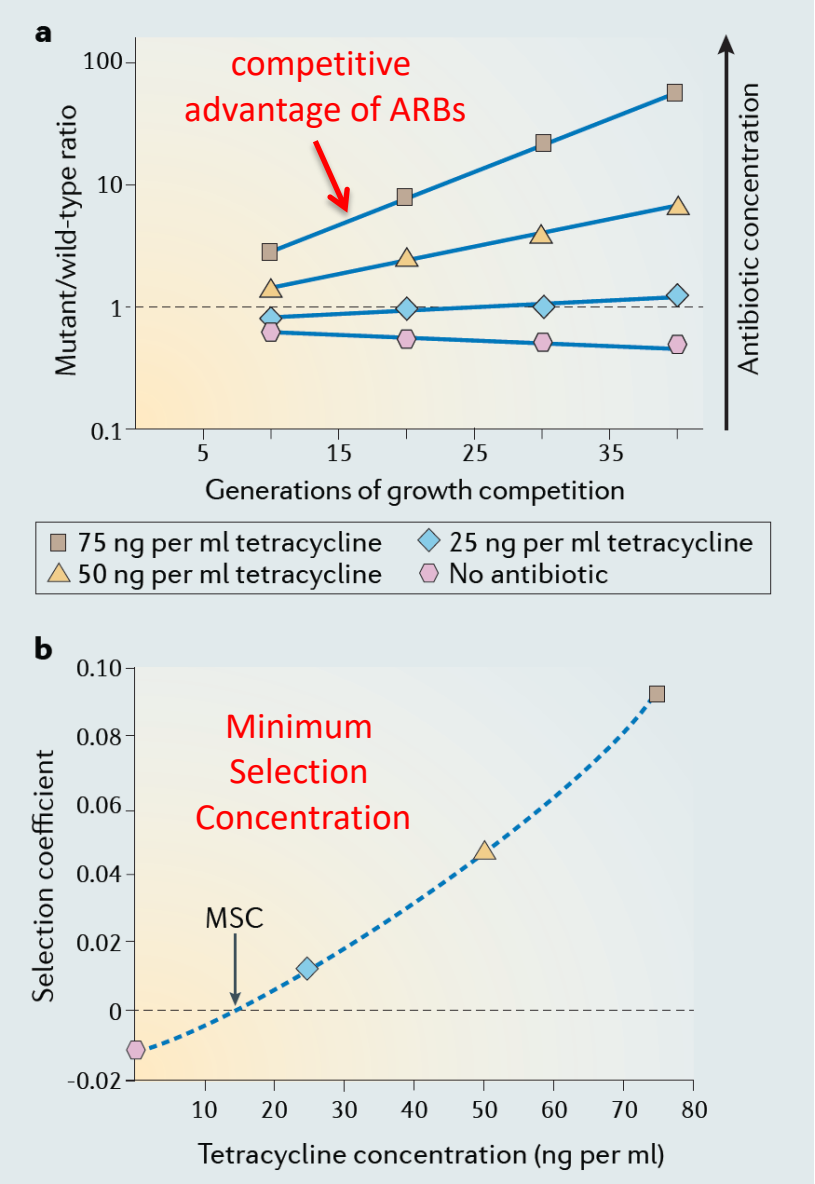
Selection of antibiotic resistant microbes (ABRs)

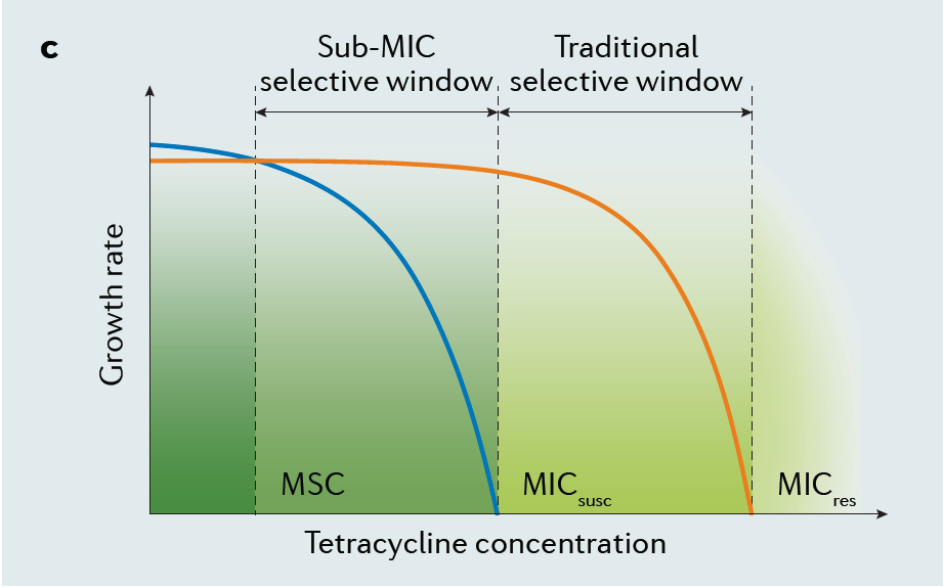
Reduction of microbial diversity

Selective advantage

(clonal expansion)

Selection of ARBs by antimicrobials at sub-MIC





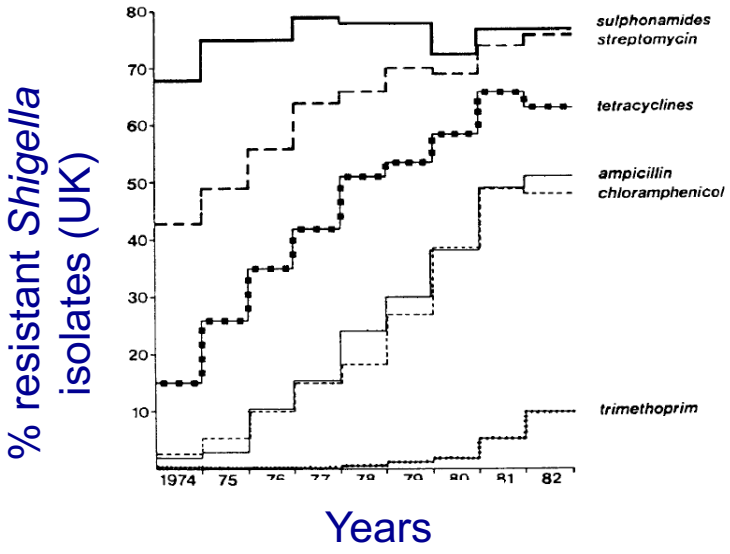
Experiment:

- mix ARBs and wild type (WT)
- allow to grow with sub-MICs of TET
- measure ARB/WT ratio overtime
- calculate selection coefficient (slope)
- plot selection coefficient against [TET]
- highest [TET] with no advantage = **MSC**

Andersson & Hughes (2014) Nat.Rev.Microbiol.

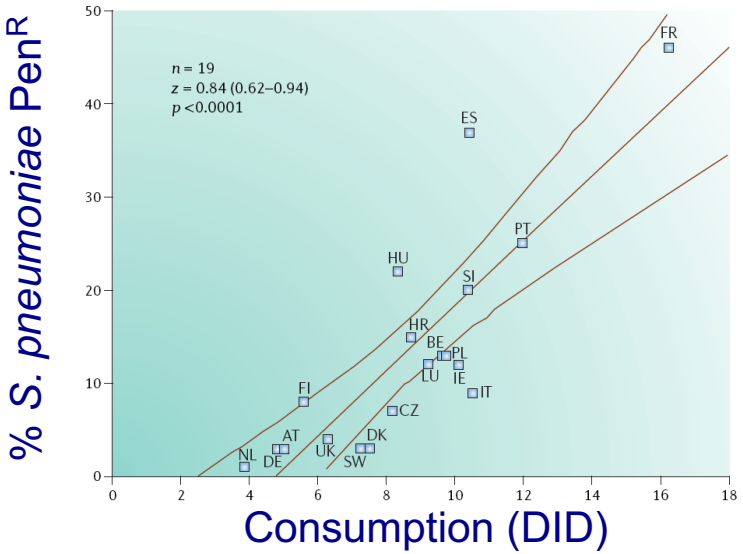
Ab consumption and Ab resistance ...
a matter of adaptation to changing environment

Occurrence of resistances to Ab increases with time



Mazel & Davies (1999) Cell Mol Life Sci.

Resistances occurrence increases with Ab consumption



Furuya & Lowy (2005) Nat. Rev. Microbiol.

Emergence de résistances aux antibiotiques chez *Staphylococcus aureus*

« Burden » : impact calculé en termes de coût financier, de morbidité ou de mortalité.

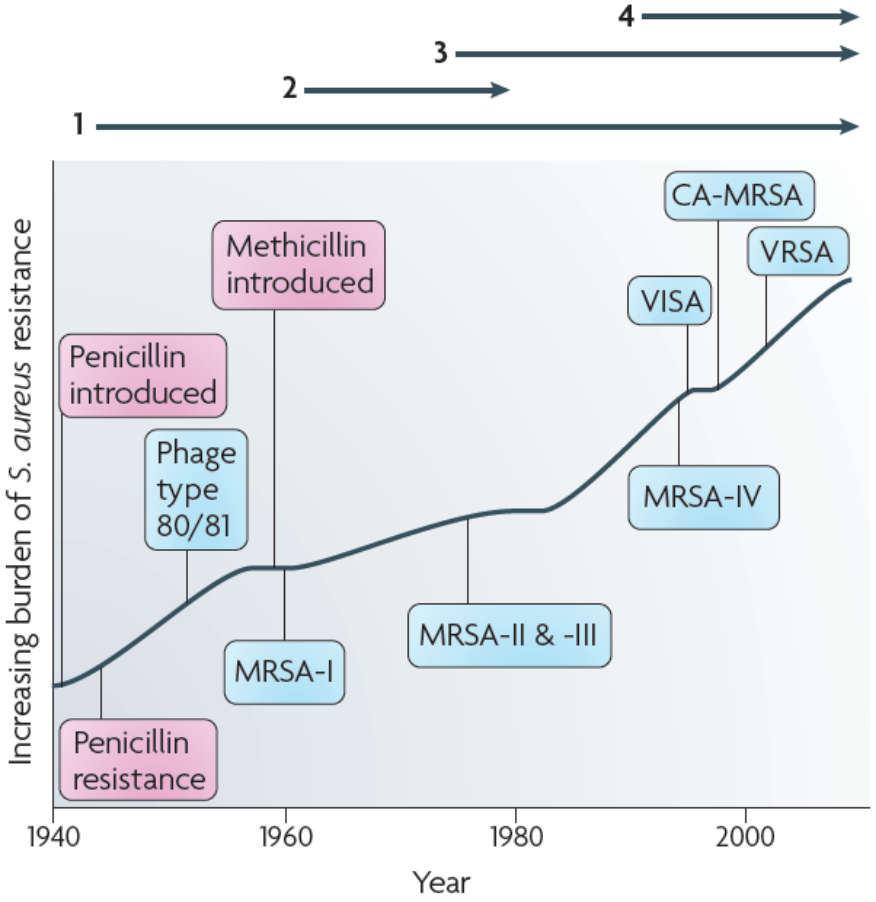
1.1. Apparition progressive de *S. aureus* Pen^R.

1.2. Première pandémie de la souche 80/81 (1957-60).

2. Emergence de MRSA-I « clone archaïque » + apparition de poly-résistances (1960-80)

3. Emergences de nouveau allotype : MRSA-II, -III et VISA (depuis 1970)

4. Apparition de MRSA communautaire (CA-MRSA) et de VRSA (depuis 1990)



Chambers & DeLeo (2009) Nat. Rev. Microbiol. 7:629-641

Pour en savoir plus : Grundmann *et al* (2006) Lancet 368:874-885

HIGH-LEVEL MEETING ON ANTIMICROBIAL RESISTANCE

21 SEPTEMBER 2016, UN HEADQUARTERS, NEW YORK

Key facts

- Antimicrobial resistance (AMR) threatens the effective prevention and treatment of an ever-increasing range of infections caused by bacteria, parasites, viruses and fungi.
- AMR is an increasingly serious threat to global public health that requires action across all government sectors and society.
- Without effective antibiotics, the success of major surgery and cancer chemotherapy would be compromised.
- The cost of health care for patients with resistant infections is higher than care for patients with non-resistant infections due to longer duration of illness, additional tests and use of more expensive drugs.
- Globally, 480 000 people develop multi-drug resistant TB each year, and drug resistance is starting to complicate the fight against HIV and malaria, as well.

What is antimicrobial resistance?

Why is antimicrobial resistance a global concern?

What accelerates the emergence and spread of antimicrobial resistance?

(World Health Organization : http://www.who.int/mediacentre/factsheets/fs194/en/)

L’émergence et la dissémination de la résistance aux antibiotiques

Mutations

(génèrent de la diversité de gènes)

Elements Genetiques Mobiles

(partagent des gènes entre micrroorganismes)

Mutation rate in bacteria

Genome size (b ou bp)

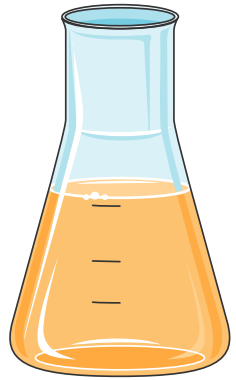
Mutations per bp per replication

Mutations per genome per replication

TABLE 3. Mutation Rates in Microbes with DNA Chromosomes[1,19]

Organism	G^a	$\mu_b{}^b$	$\mu_g{}^c$
Bacteriophage M13	6.4×10^3	7.2×10^{-7}	0.0046
Bacteriophage λ	4.9×10^4	7.7×10^{-8}	0.0038
Bacteriophages T2 and T4	1.7×10^5	2.4×10^{-8}	0.0040
Escherichia coli	4.6×10^6	5.4×10^{-10}	0.0025
Saccharomyces cerevisiae	1.2×10^7	2.2×10^{-10}	0.0027
Neurospora crassa	4.2×10^7	7.2×10^{-11}	0.0030
Mean			0.0034

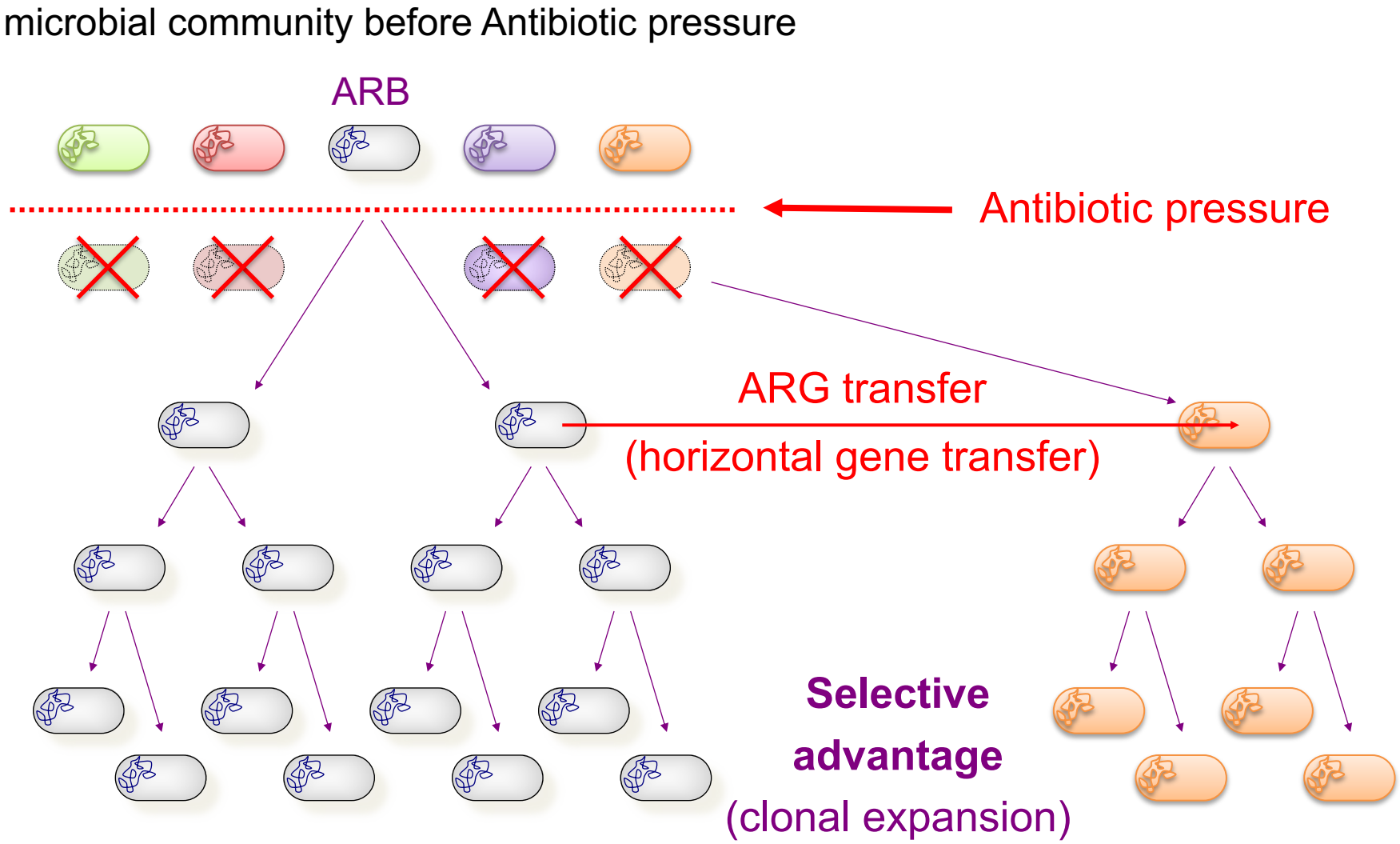
Drake (1999) Ann. N. Y. Acad. Sci. 870:100-107



1 mutant every 400 cells

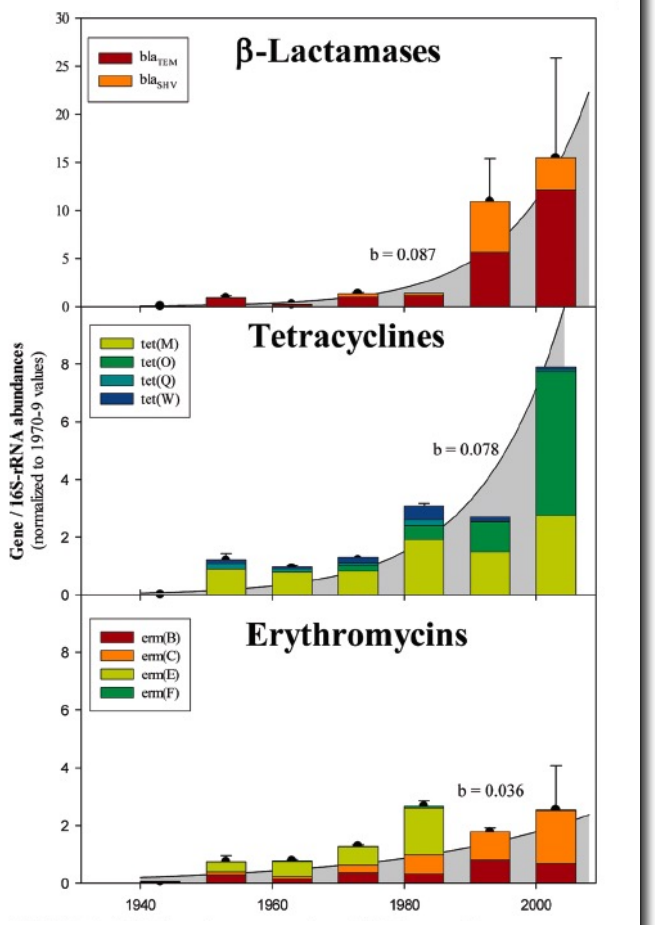
1 mutant for each nucleotide in a 1 mL culture of *E. coli* (2.10^9 cells)

Consequences of gene transfer and a selective pressure (even moderate)



Evidences of gene transfer in the environment

AbR gene abundances in archived soils since 1940



Knapp *et al.* (2010) E.S.T. 44:580–587

Direct evidences.

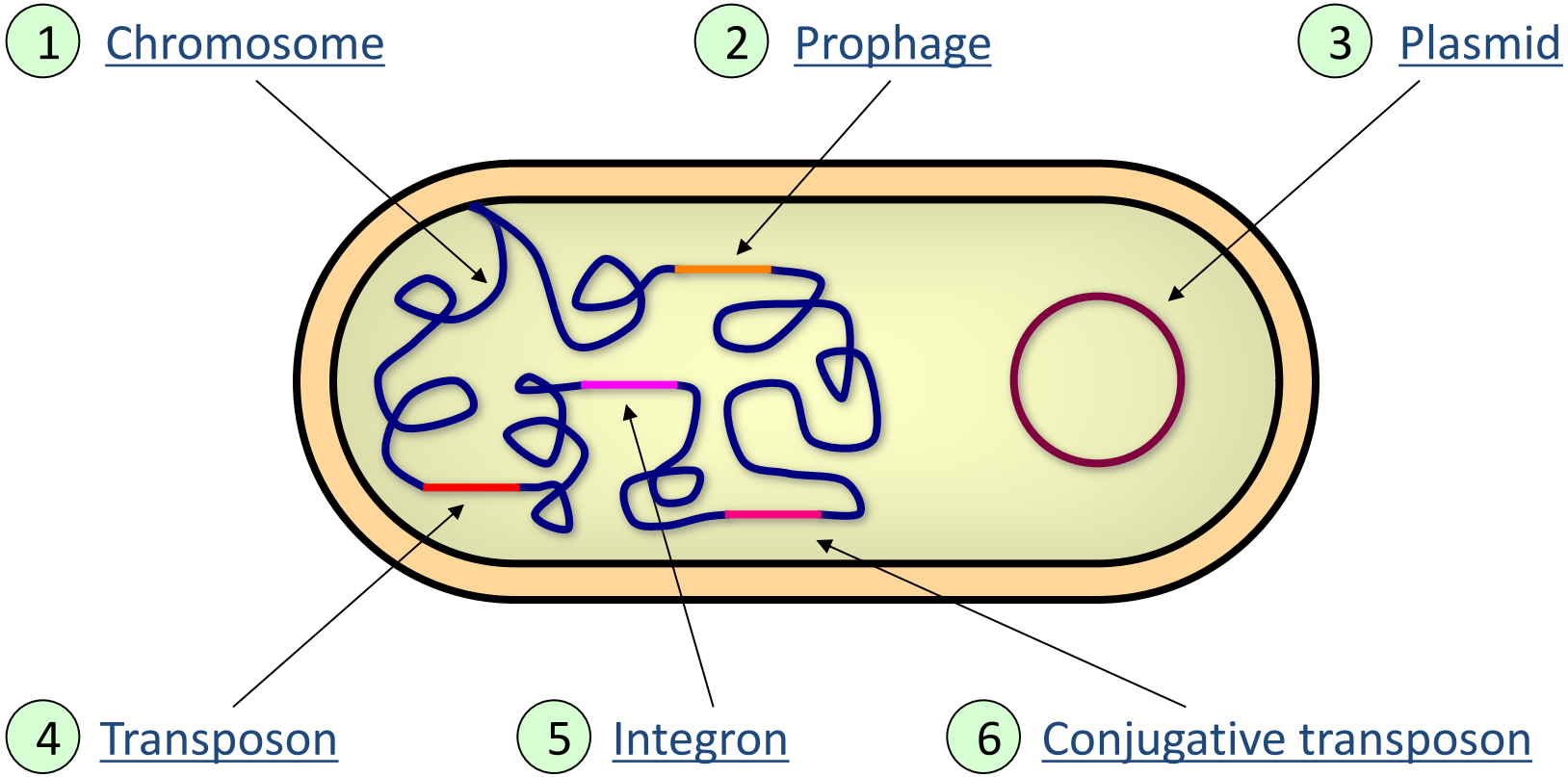
- Rare, difficulty to distinguish between transfer and clonal amplification.

Retrospective evidences.

- Absence of congruence between the phylogeny of the transferred genes and that of 16S rDNA.
- Loss of synteny at the extremity of the transferred DNA.
- Transferred genes are often associated to mobile genetic elements.

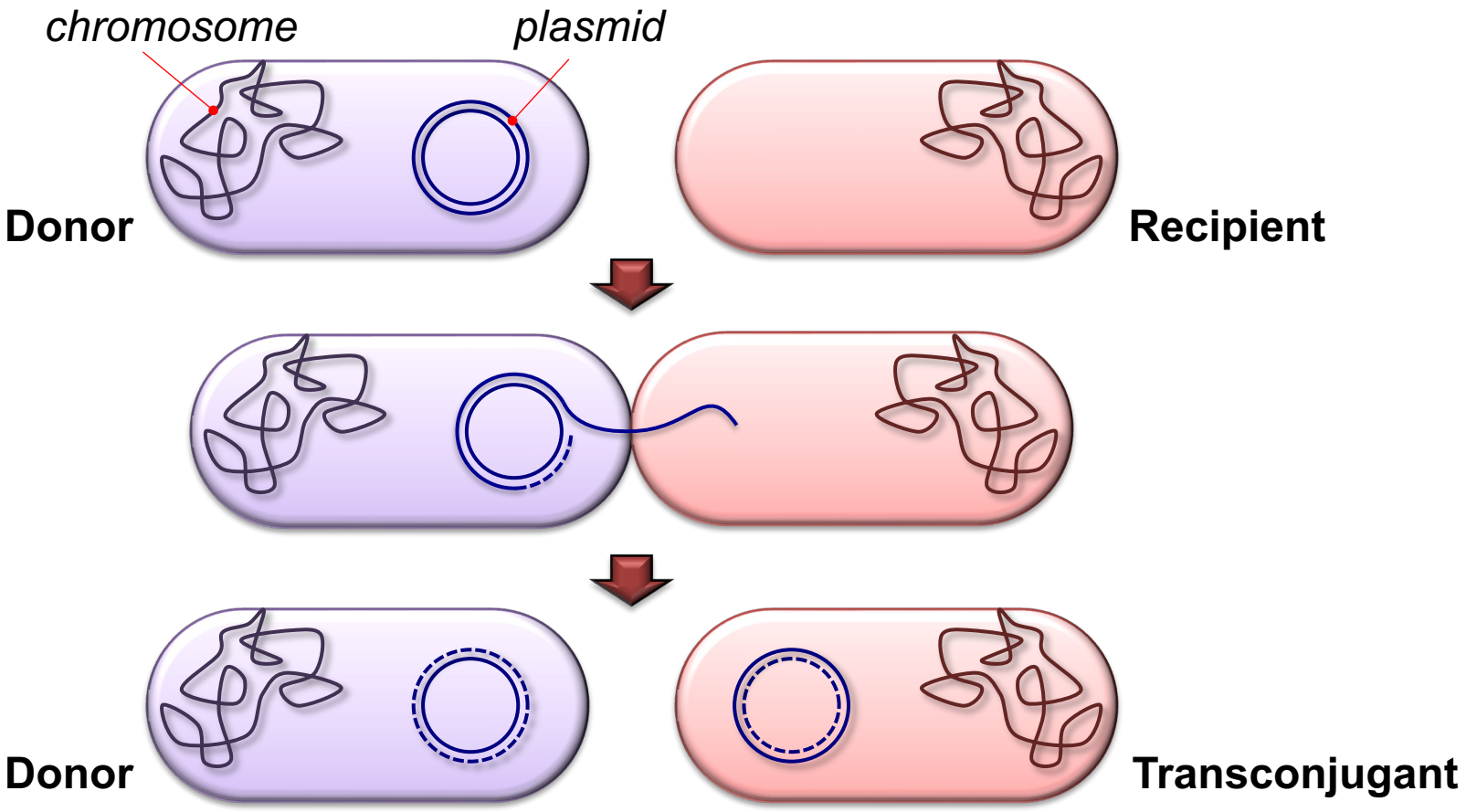
Does it take place in the environment?
What is the driving force?

Bacterial genomes and “accessory” genetic elements



Genome = core genome + flexible gene pool (unstable)

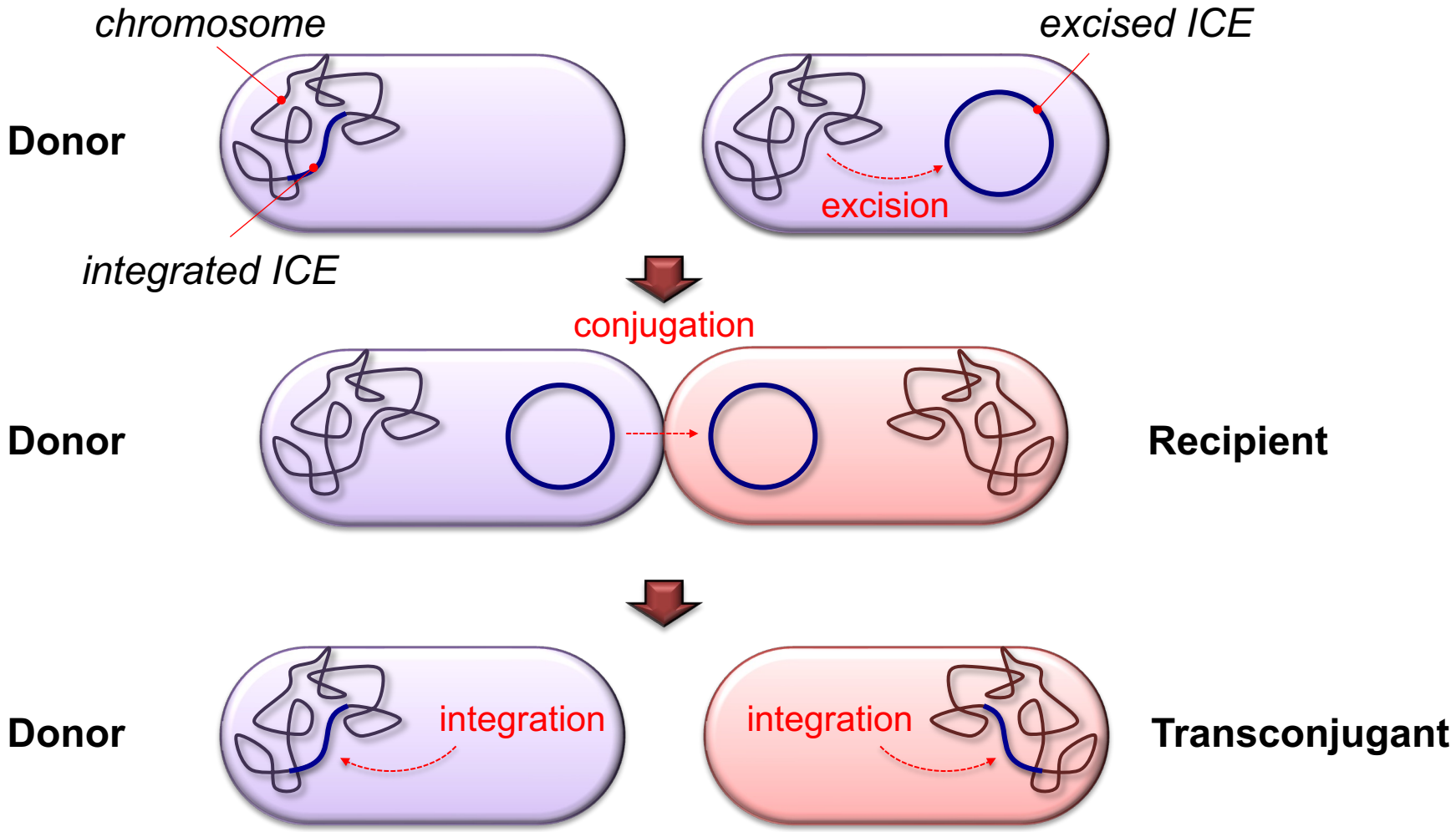
Plasmid transfer by conjugation



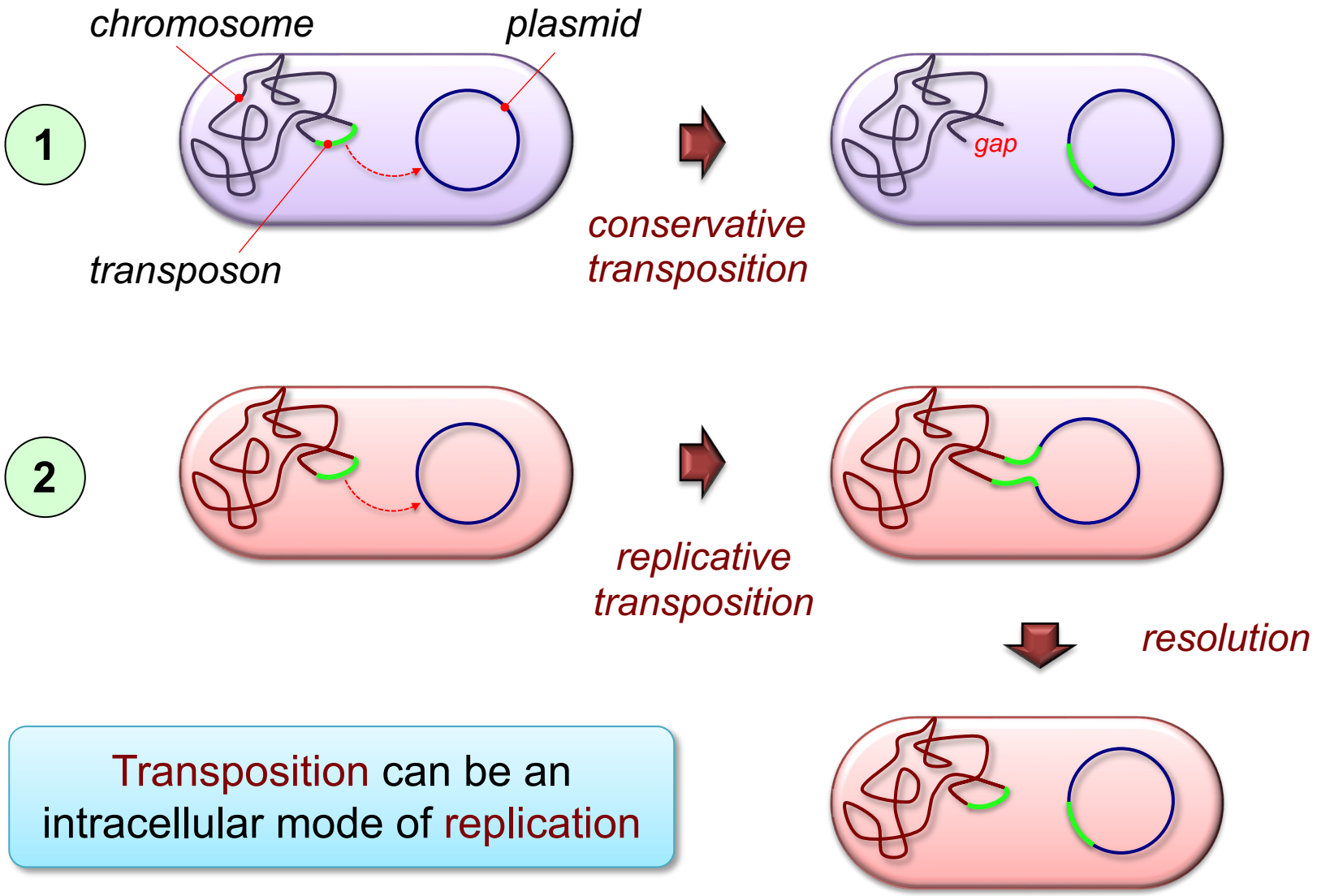
Conjugation is an intercellular mode of replication!

ICE transfer by excision & conjugation

(Integrative and Conjugative Elements)

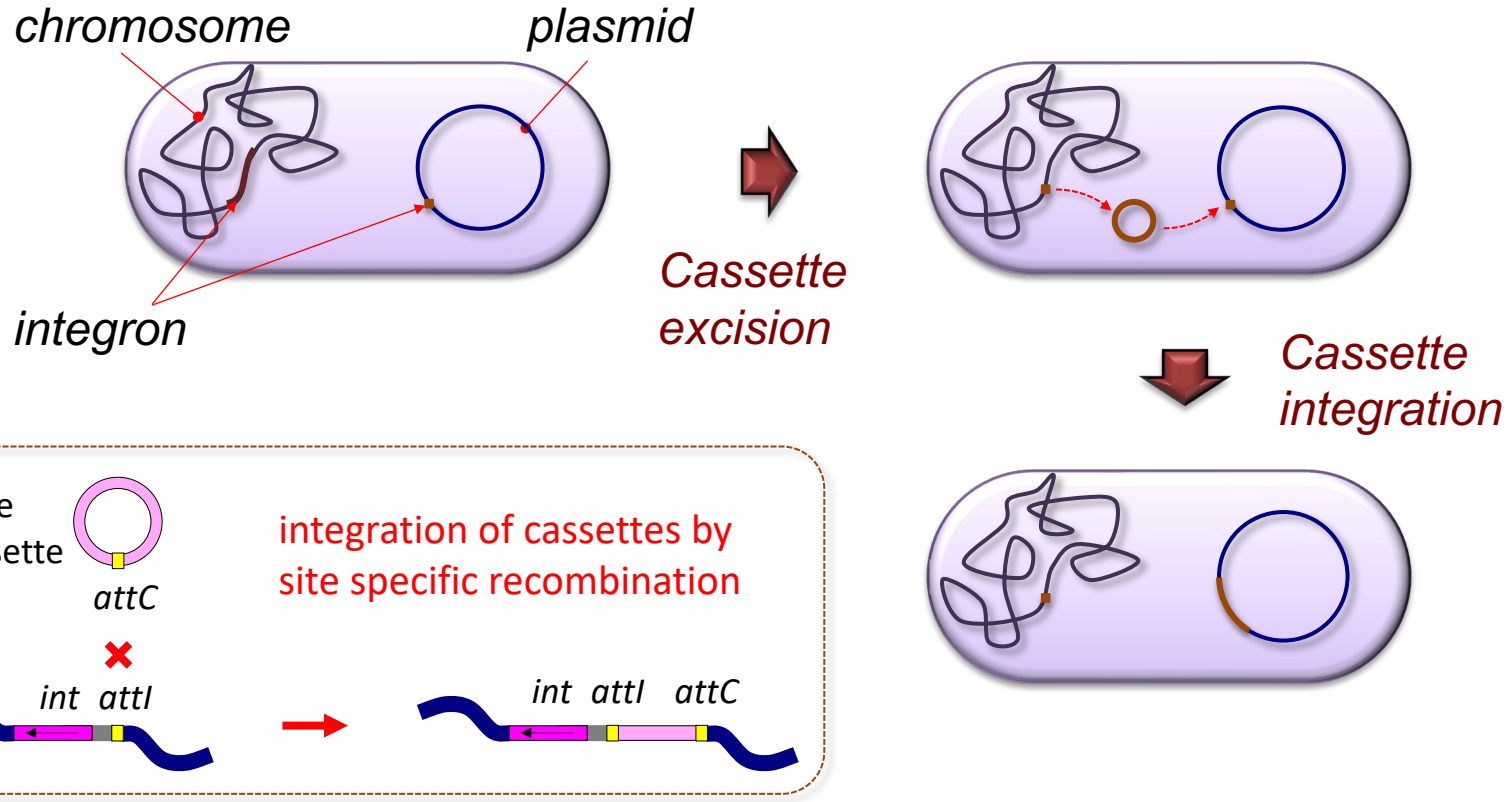


Transposition by transposable elements



Transposition can be an intracellular mode of replication

Gene cassette capture by integrons



Integrons are not mobile DNA but they can be associated to other mobile genetic elements

Genetic map of the natural plasmid pB10

- 72 genes organized in functional modules (colored arrows).
- Ubiquitous (IncP-1β family) – Isolated from activated sludge.
- Antibiotic resistances: Amx, Str, Sul, Tet.
- Transfer efficiency : up to 80% of transconjugants per recipient (plate mating).

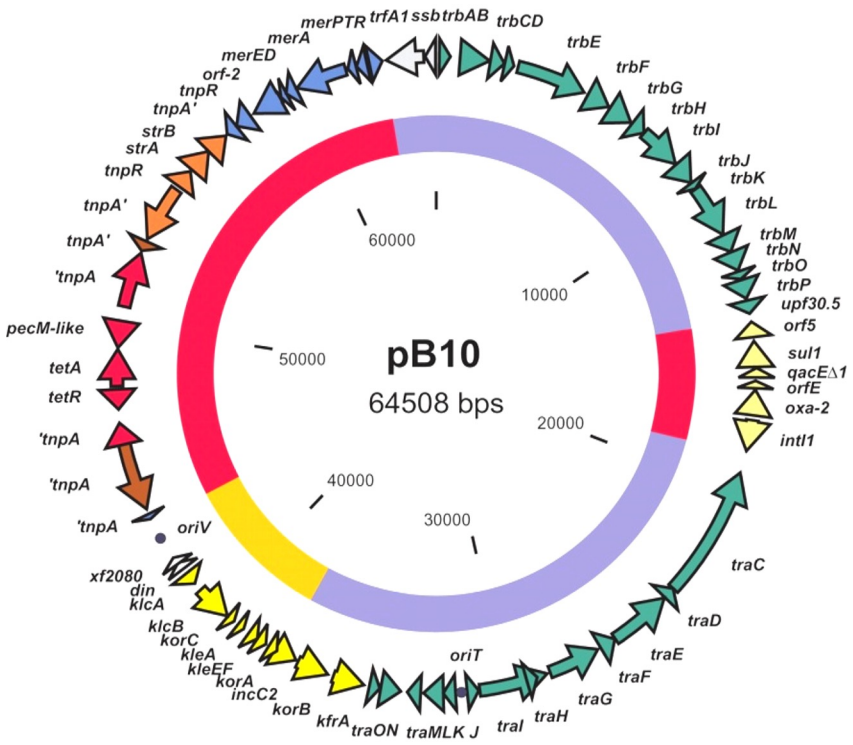
Tn*501*-like (Hg^R)

Tn*5393c* (Str^R)

Tn*1721-like* (Tet^R)

IS*1071*

Central control region



Replication

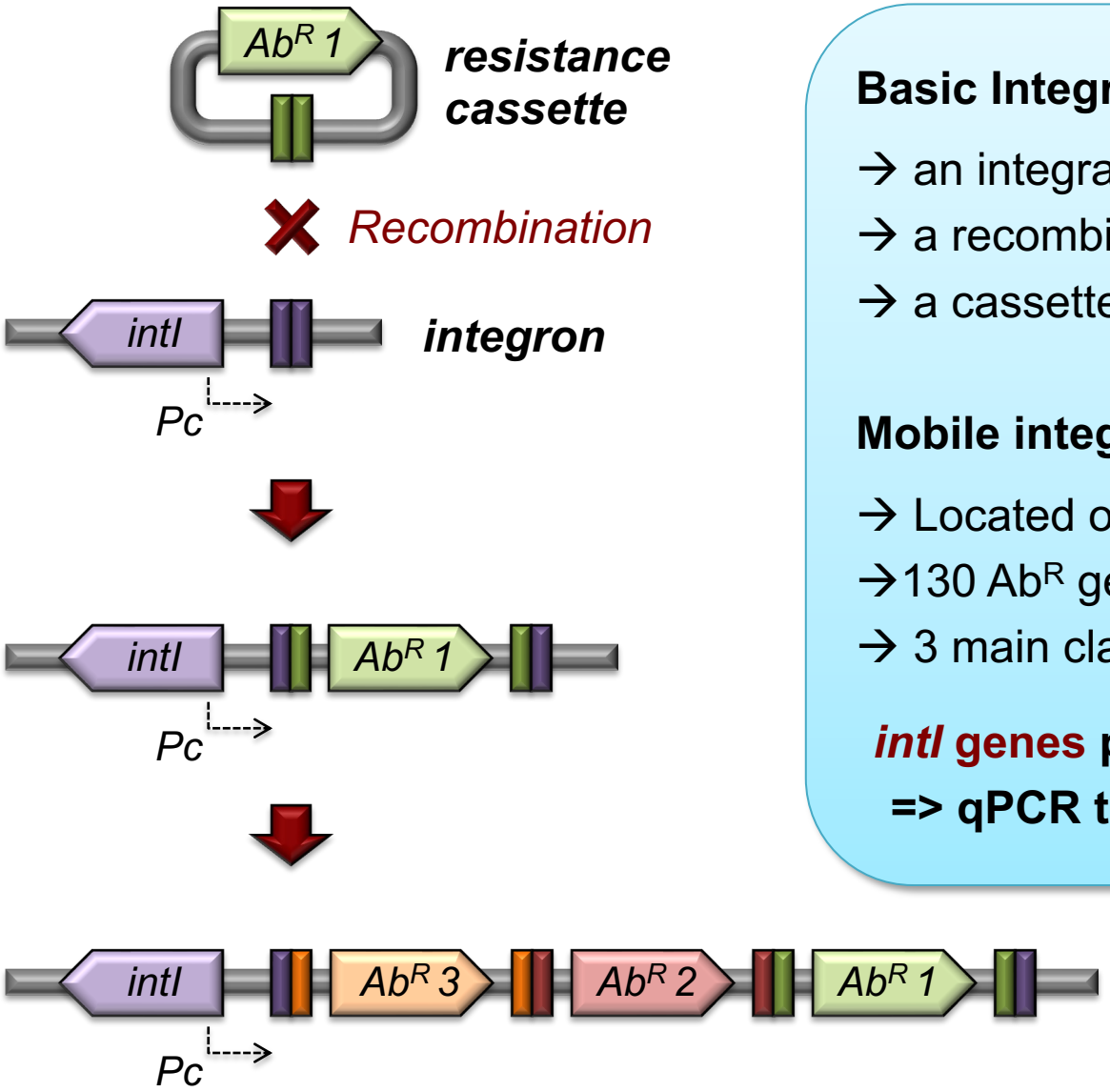
Mating-pair-formation

Class I integron (Amx^R - Sul^R)

Conjugative transfer

Schlüter *et al.* (2003)
Microbiology 149:3139-3153

Integrons as platforms of ARGs capture



Basic Integrons platform:

→ an integrase gene: *intI*

→ a recombination site: *attI*

→ a cassette promoter: Pc

Mobile integron (MI):

→ Located on mobile DNA elements

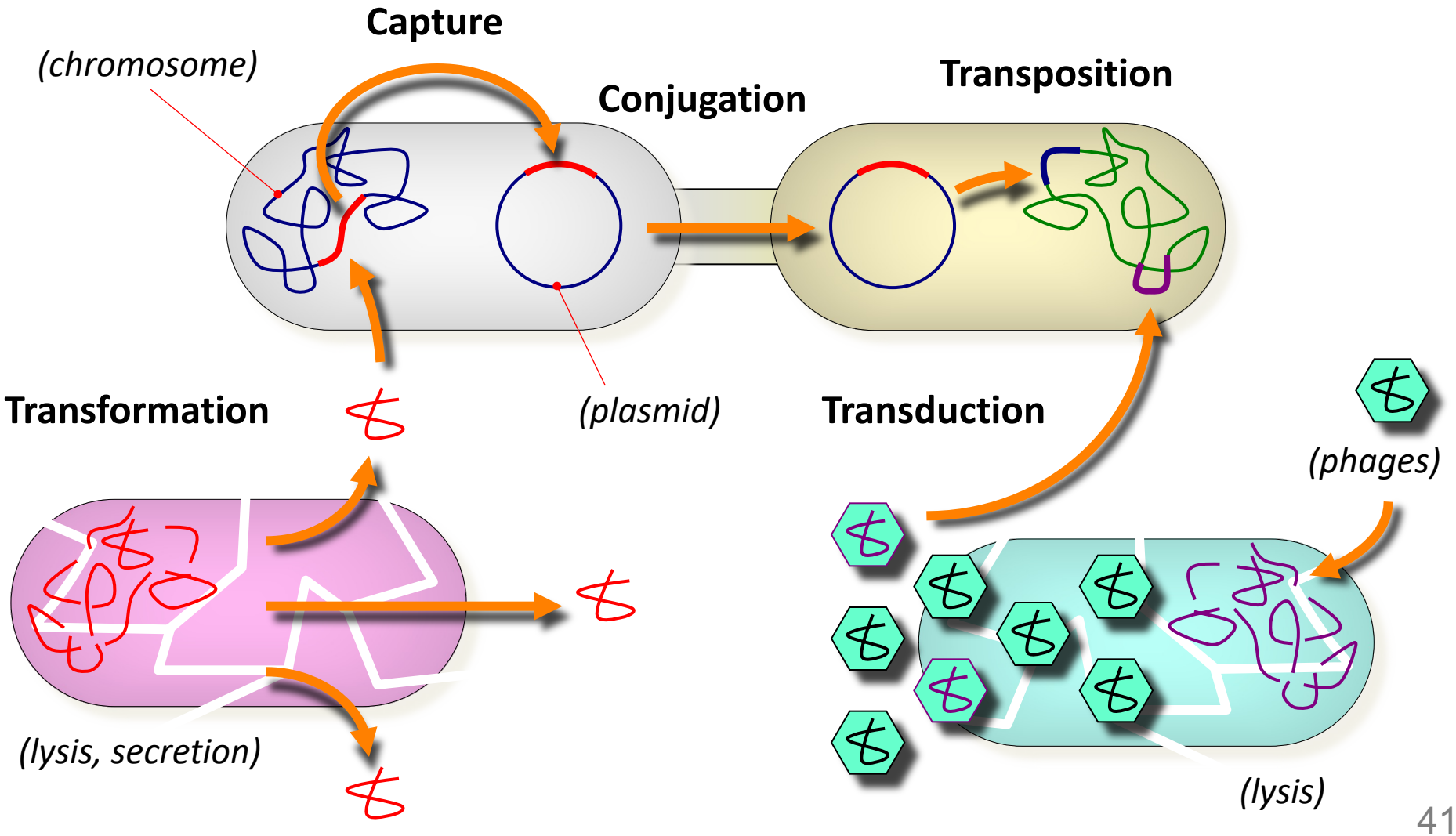
→130 Ab^R gene cassettes described

→ 3 main classes of MI described

***intI* genes present on all integrons**

=> qPCR target (proxy for ARGs)

Genetic instability and gene acquisition in bacteria



Contrôle de la résistance aux antibiotiques

...effects des antibiotiques à basses concentrations

The antibiotic context

- ≈ **3000** pharmaceutical compounds currently used in Europe (painkillers, antibiotics, antidiabetics, beta-blockers, contraceptives, lipid regulators, antidepressants, antineoplastics, tranquilizers, impotence drugs and cytostatic agents)

- **12 500** tons of antibiotics consumed each year in Europe

consumption

partial transformation

excretion

wastewater treatment plants

Impact?

effluents

2010

Fate of antibiotics

an example with sulfamethoxazole

annual consumption:

- EU : 12,500 t. **(200 t.)**
- USA : 16,000 t. **(300 t.)**

consumption
partial transformation
excretion

Environment

Soil: 0.01 to 10 µg/Kg
Water: 1 to 200 ng/L

WWTP

0.1 to 2 µg/L

50 to 100 ng/L

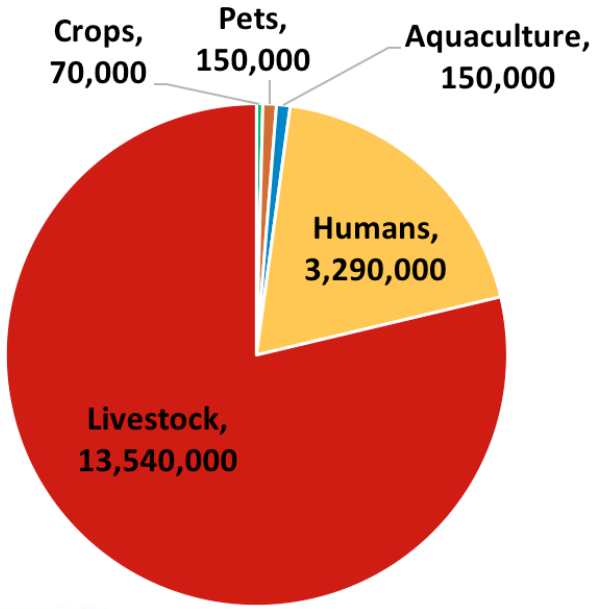
100 to 290 ng/L

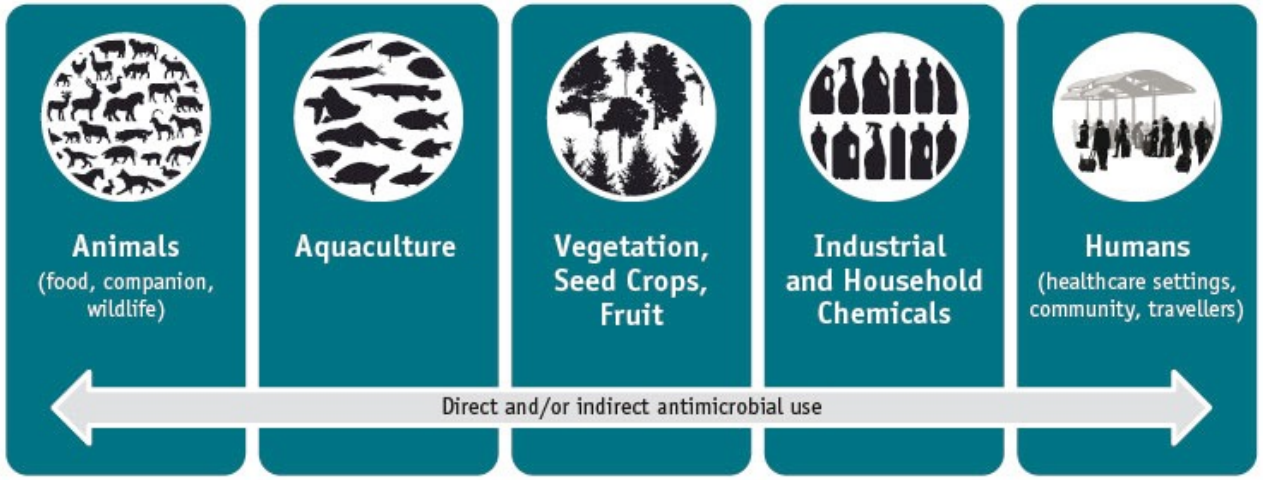
Effects?

2010

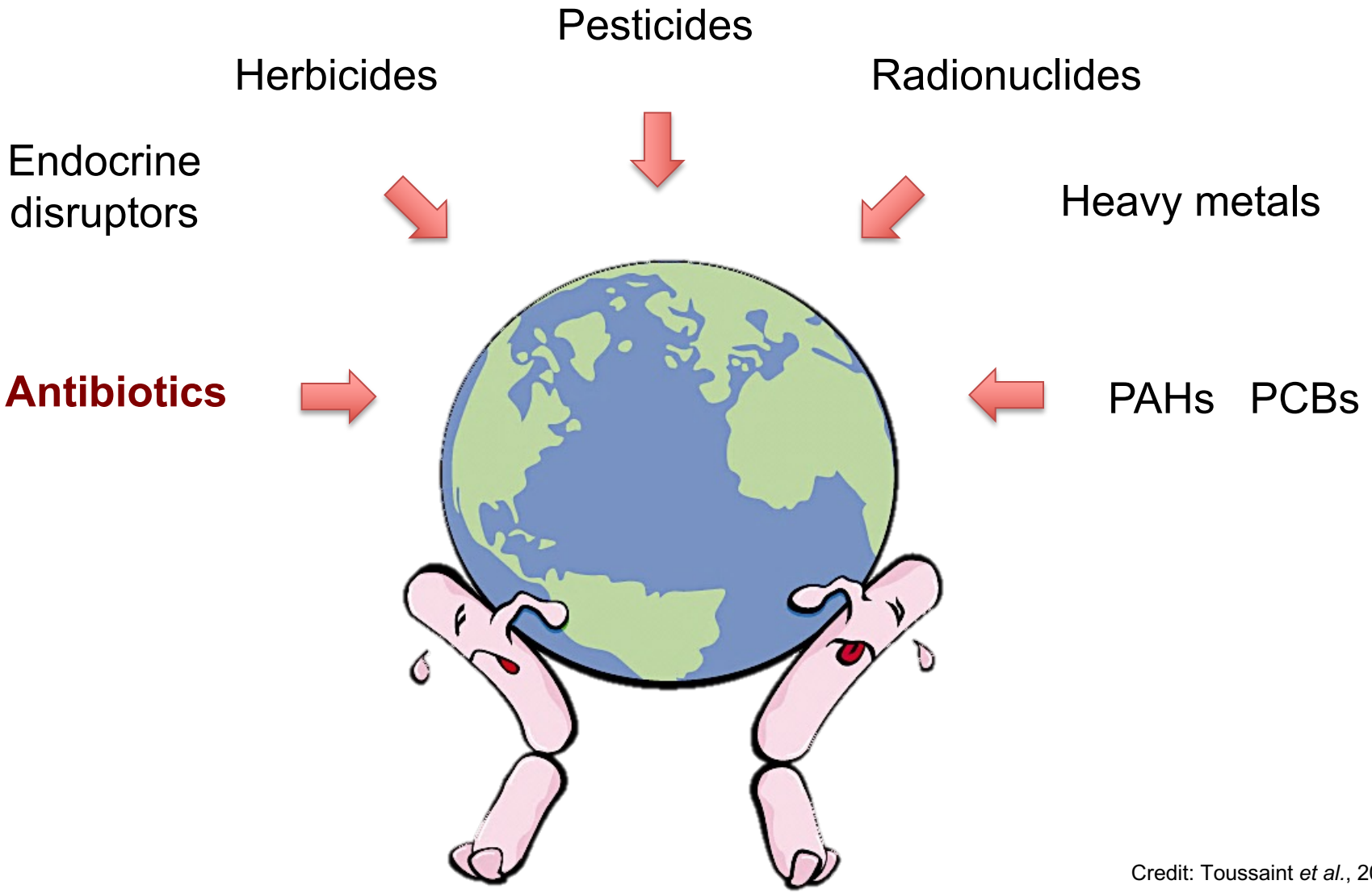
Annual antibiotic consumption in the USA (kg)

Antibiotics are not only used for health but also as growth promoting agents





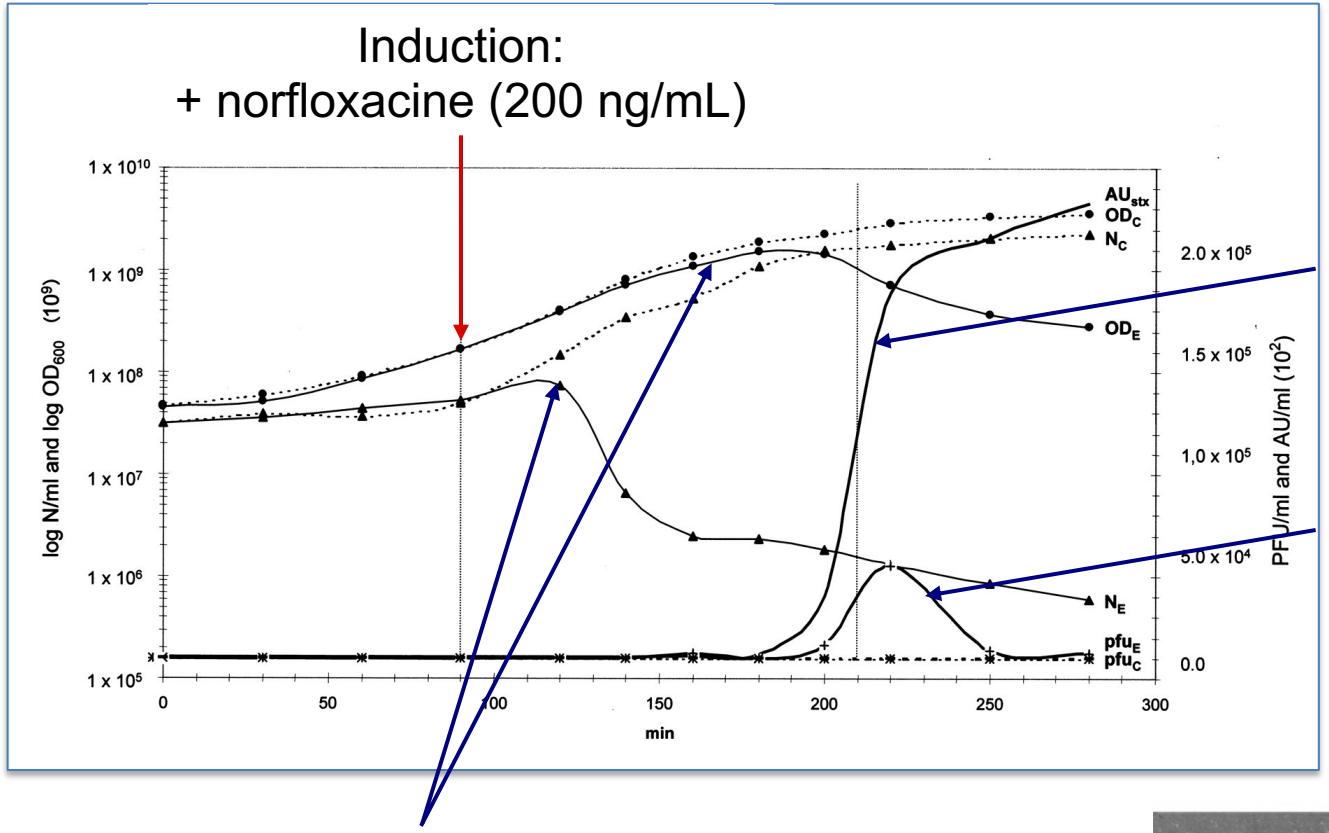
On an environmental point of view microbes are our life-support system



Credit: Toussaint *et al.*, 2003

Influence of norfloxacin on the virulence of bacterial pathogens

(*E. coli* O157 EDL933)

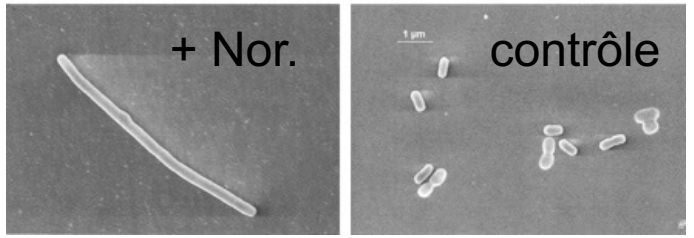


2 Production of shigatoxin STX

3 induction of phage (pfu/mL)

1 Lower cell count (cfu/mL) but increasing biomass

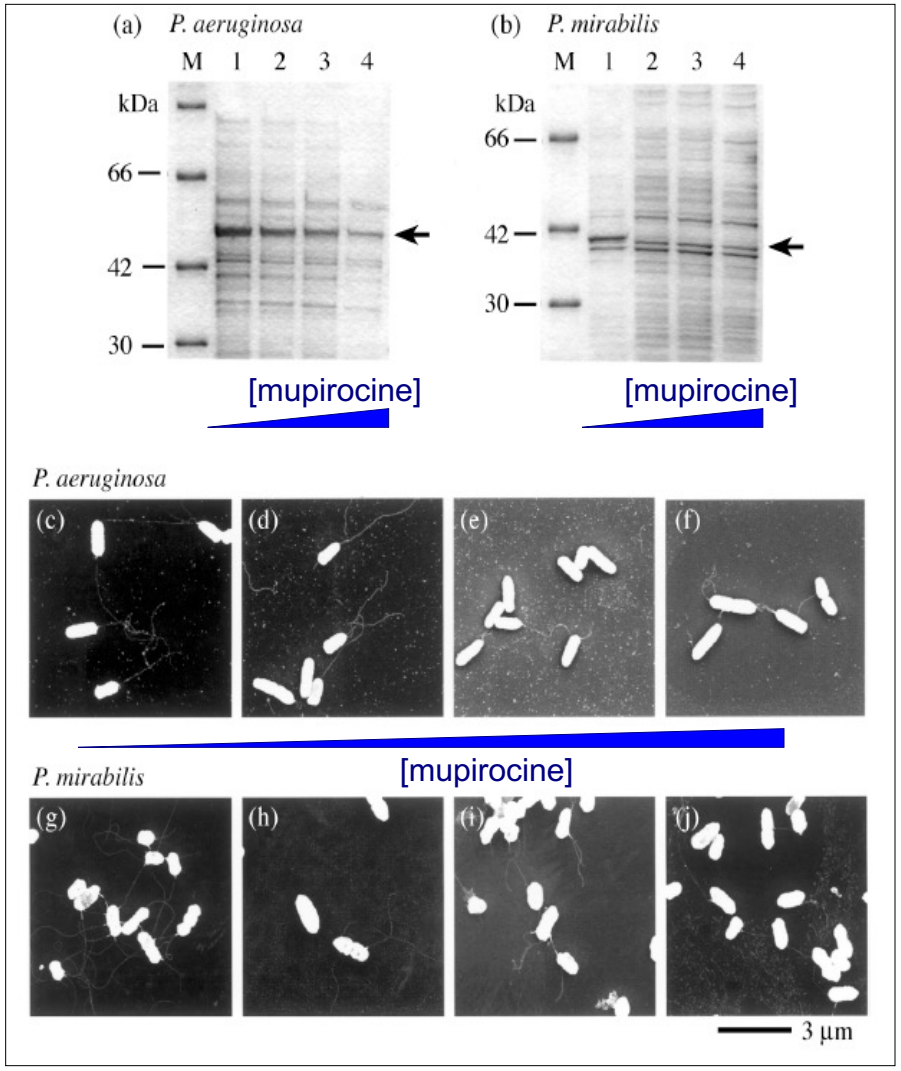
(filamentation)



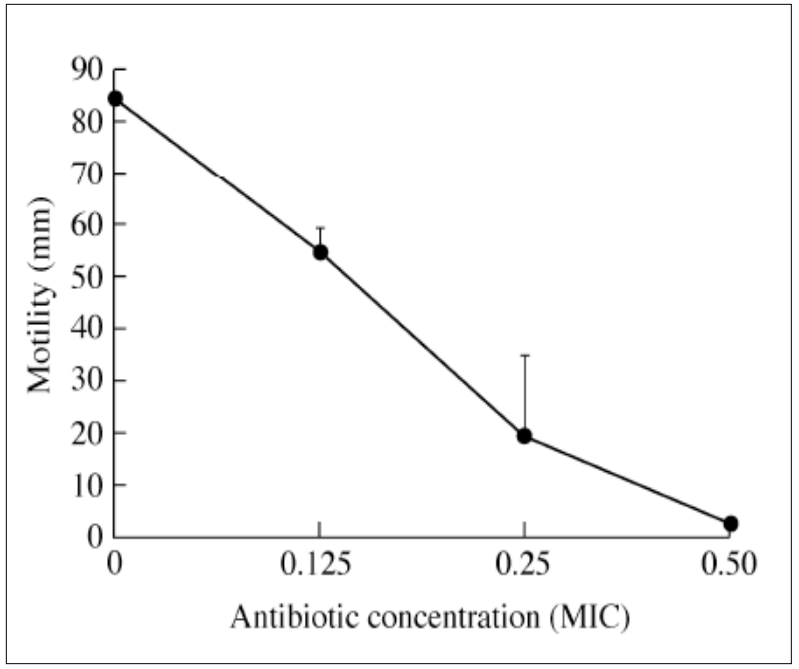
(Control = no antibiotics)

Herold et al. (2005) AAC. 49(3):931-944

Effect of mupirocine at sub-inhibitory concentrations on bacterial motility



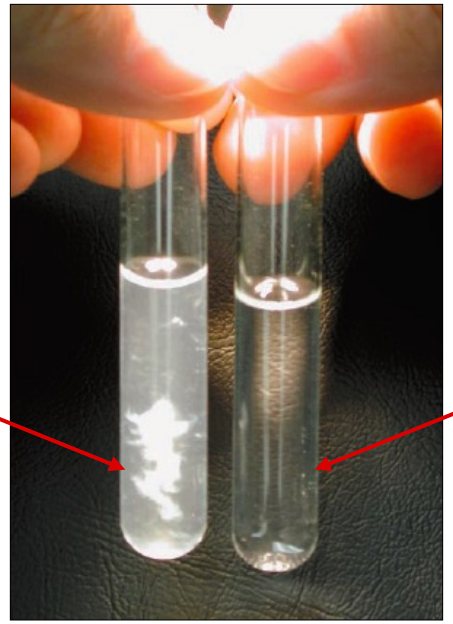
- lower flagellin synthesis
- alteration of flagella formation
- lower mobility (*P. mirabilis*)



Horii et al. (2003) Journal of Antimicrobial Chemotherapy. 51:1175–1179

Effects of antibiotices on the production of exopolymeric substances in *P. aeruginosa*

Biofilm of *P. aeruginosa*
+ imipenem during 24 h.
⇒ Alginate x 20 (matrix).



(precipitation of exopolysaccharides from biofilms)

Bagge et al. (2004) AAC. 48(4):1175-1187

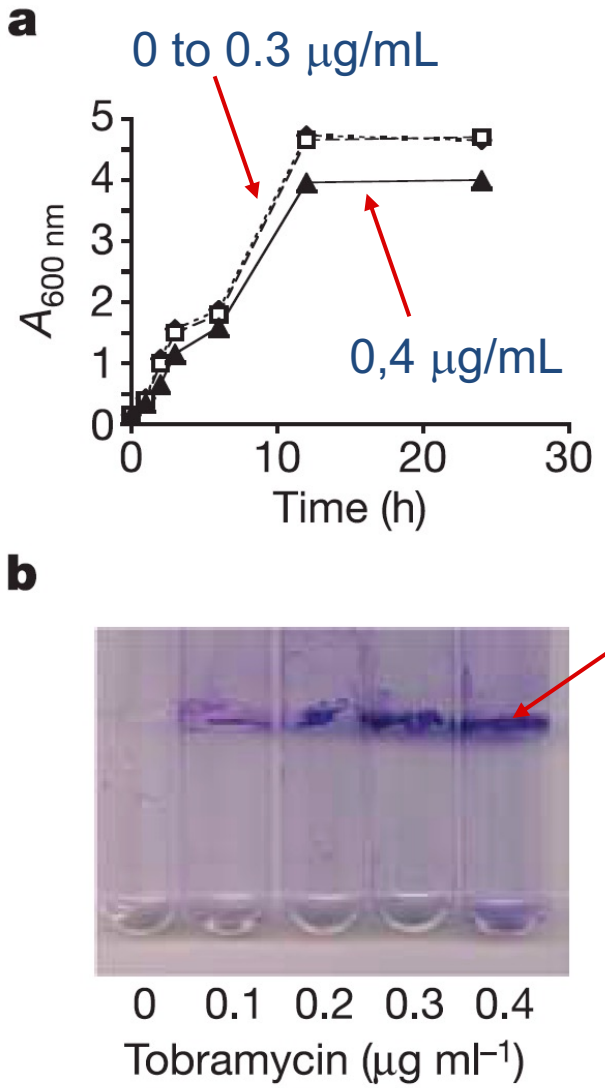
Effect of some antibiotics on the expression of the *ica* genes *(coding for extracellular polysaccharides)*

TABLE 2. MICs of different antibiotics and effects of sub-MICs on ica_{prom}::*lacZ* expression

Antibiotic	MIC (µg/ml) for *S. epidermidis* 220	Effect of sub-MICs on ica_{prom}::*lacZ* expression in *S. epidermidis* 220-1
Penicillin G	0.1	No effect
Oxacillin	0.125	No effect
Chloramphenicol	0.25	No effect
Clindamycin	0.25	No effect
Erythromycin	70	2.5-fold increase
Gentamicin	1	No effect
Quinupristin-dalfopristin	0.5	11-fold increase
Tetracycline	0.5	9-fold increase
Ofloxacin	0.5	No effect
Vancomycin	1	No effect
Teicoplanin	0.125	No effect

Rachid et al. (2000) AAC. 44(12):3357-3363

Influence of tobramycin on biofilm formation



1. The growth of *P. aeruginosa* is not impaired at subinhibitory concentration of tobramycin.

(MIC = 1 μg/mL)

2. The antibiotics increases the formation of biofilms.

(biomass stained with crystal violet)

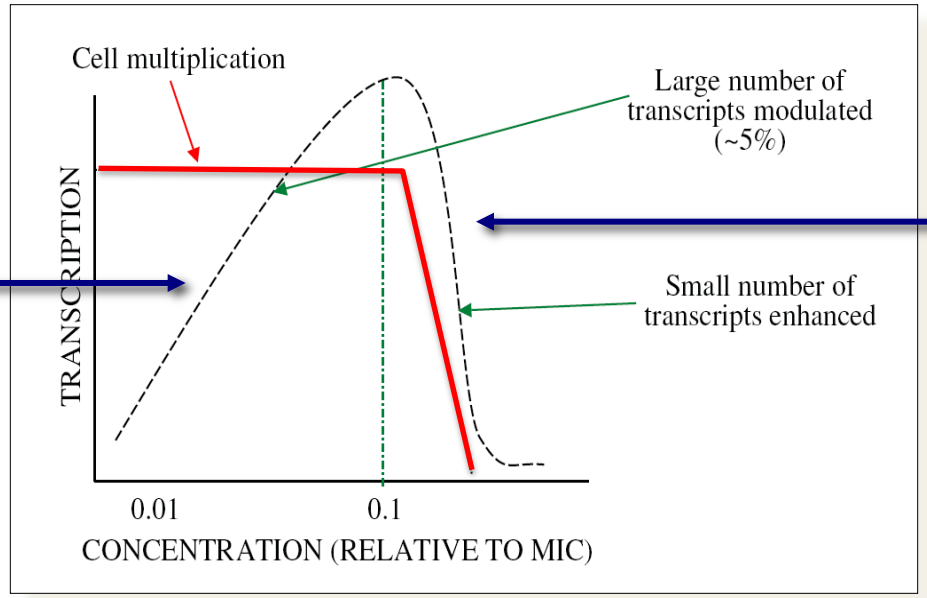
Hoffman et al. (2005) Nature. 436:1171-1175

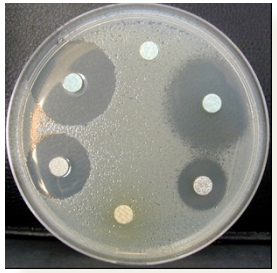
Antibiotics and hormesis

Hormesis: biphasic dose-response relationship

(stimulation at low concentrations & inhibition at elevated concentrations)

environmental conditions



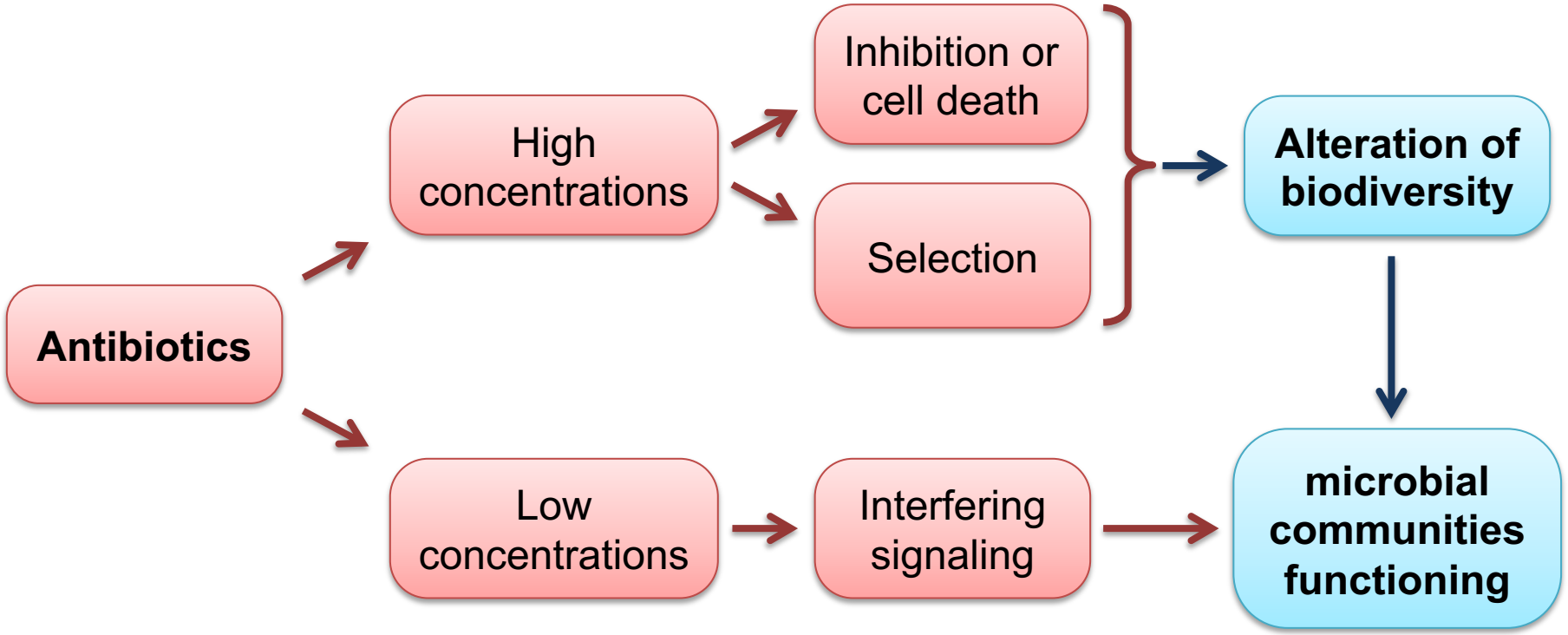


therapeutic conditions

Modulation of : - EPS production - Biofilm formation - Quorum sensing
- Virulence - Induction of gene transfer.

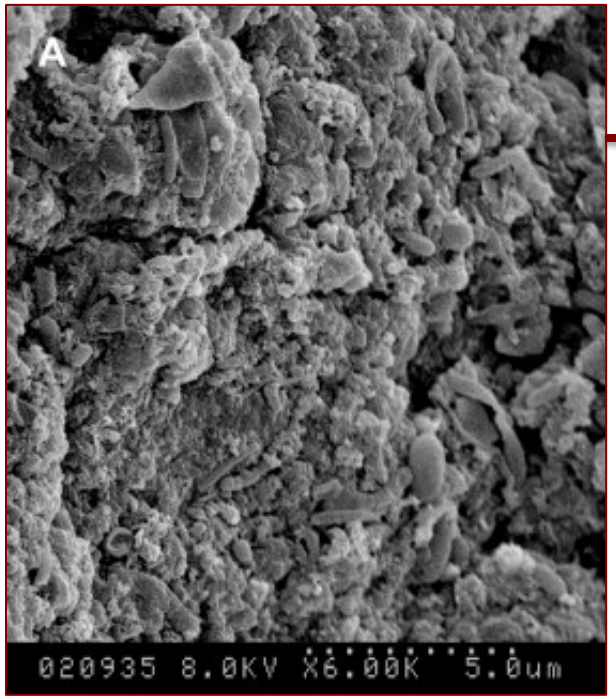
Yim et al. (2006) Int. J. Med. Microbiol. 296:163-170

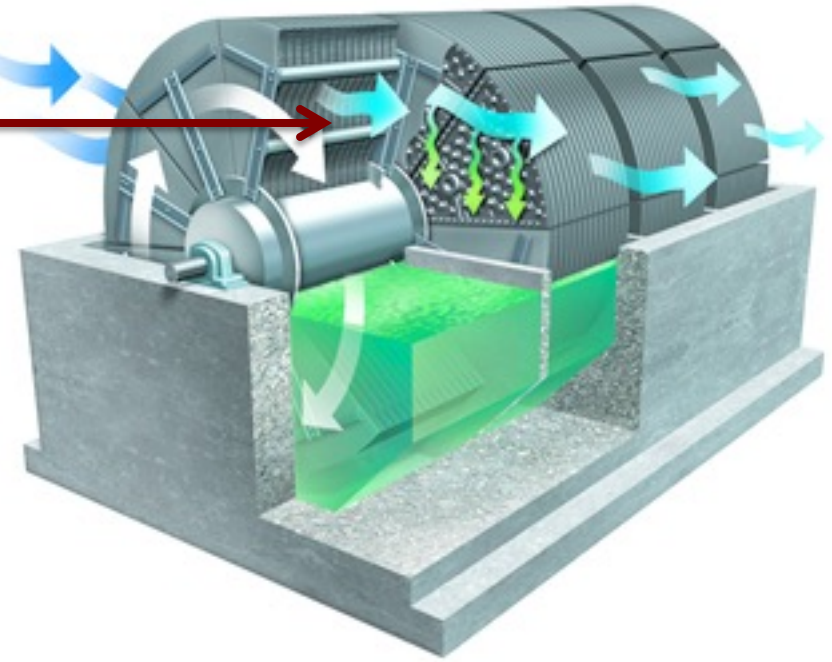
Effects of antibiotics at high and Low concentrations



Biofilms are everywhere and involved in numerous processes could antibiotics interfere with the process?

Biofilms in rotating biological contactors of WWTP





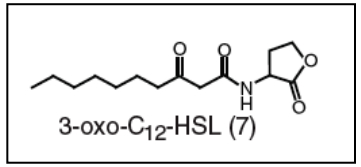
Sheng and Liu (2011)
Water Research
45(18) 6039–6050

http://www.walker-process.com/prod_bio_RBC.htm

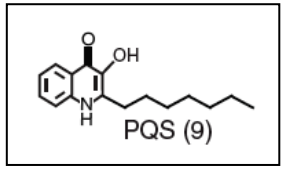
Quorum sensing regulatory network in *Pseudomonas aeruginosa*

***P. aeruginosa* growth**

Build-up of 3O-C12-HSL



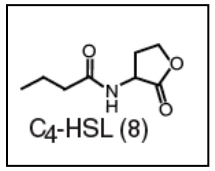
Build-up of PQS (quinolone)



Virulence genes (+)

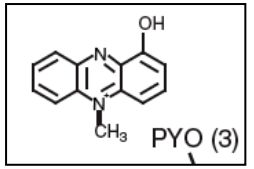
- elastase
- exotoxin
- alkaline protease
- C4-HSL & PQS synthesis

Build-up of C4-HSL



(-)

Synthesis of Pyocyanin



Virulence genes (+)

- Rhamnolipids

Disruption of cell membranes - disruption of bronchial epithelium - paracellular invasion...

(+)

Biofilm

Pyocyanin as a signaling molecule for the formation of *Pseudomonas aeruginosa* biofilm

Biofilm of *P. aeruginosa* at day 4 (flow cell)

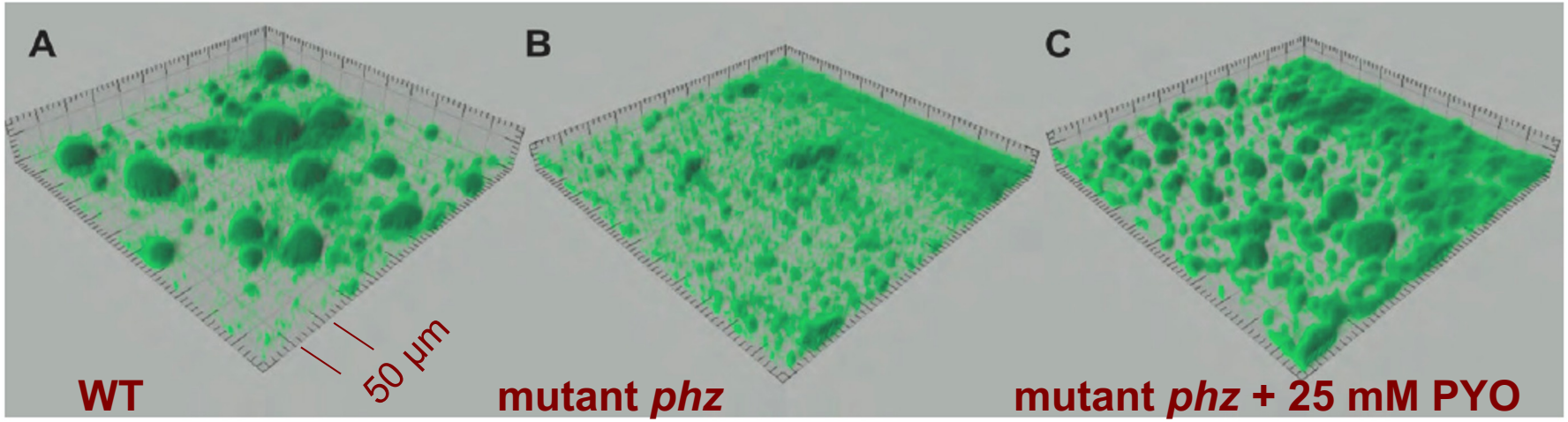
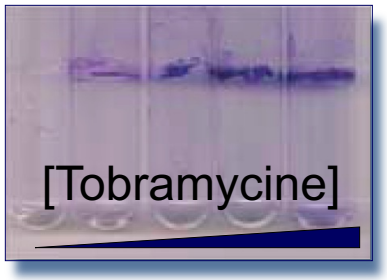


Fig. 2. Phenazines support development of structured biofilms in a continuous flow cell system. Representative images of 4-day-old biofilms of the wild-type (A) and *phz1/2* mutant without (B) and with addition of 25 µM pyocyanin (C) to the nutrient flow. Images of similar positions in the flow cell were taken in triplicate. Three independent experiments were performed with similar results.

Ramos et al. (2010)
Res. Microbiol. xx-1-5

Antibiotics at sub-inhibitory concentrations



Hoffman *et al. (2005)*
Nature. 436:1171-1175

Bagge *et al. (2004)*
AAC. 48(4):1175-1187

Cell mobility

Biofilm formation

EPS production

Virulence

Antibiotics

Quorum sensing

global transcriptional activity

etc...

Gene transfer

Yim *et al.* (2006) International Journal of Medical Microbiology. 296:163-170

Antibiotics as stimulators of resistance gene dissemination

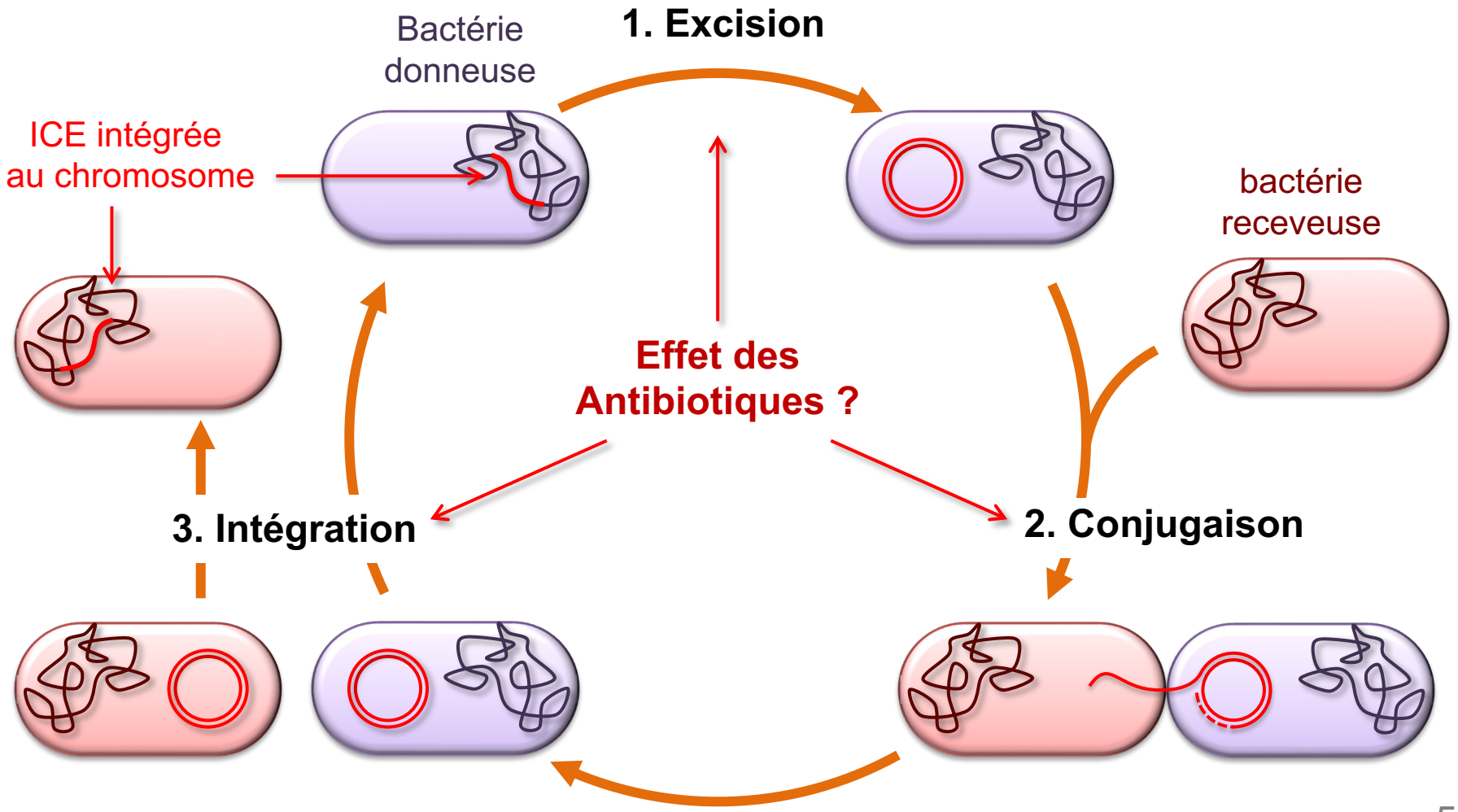
Mobile genetic elements known to be induced by sub-MIC levels of Ab

Mobiles Elements	Antibiotics	Effects / mechanisms	References
CT*nDOT* (ICE)	Tetracycline	Transfer x1000 / Attenuation	Salyers *et al.*, 1995
T*n917* (transposon)	Erythromycin	Transposition x6 / Attenuation	Clewell *et al.*, 1982
T*n1545* / Tn*916* (ICE)	Tetracycline	Transfer x20 / Attenuation	Doucet-Populaire *et al.*, 1991
SXT (ICE)	Ciprofloxacine	Transfer induced / SOS	Beaber *et al.*, 2004
Integrons	Quinolones,...	Recombination induced / SOS	Cambray *et al.*, 2011

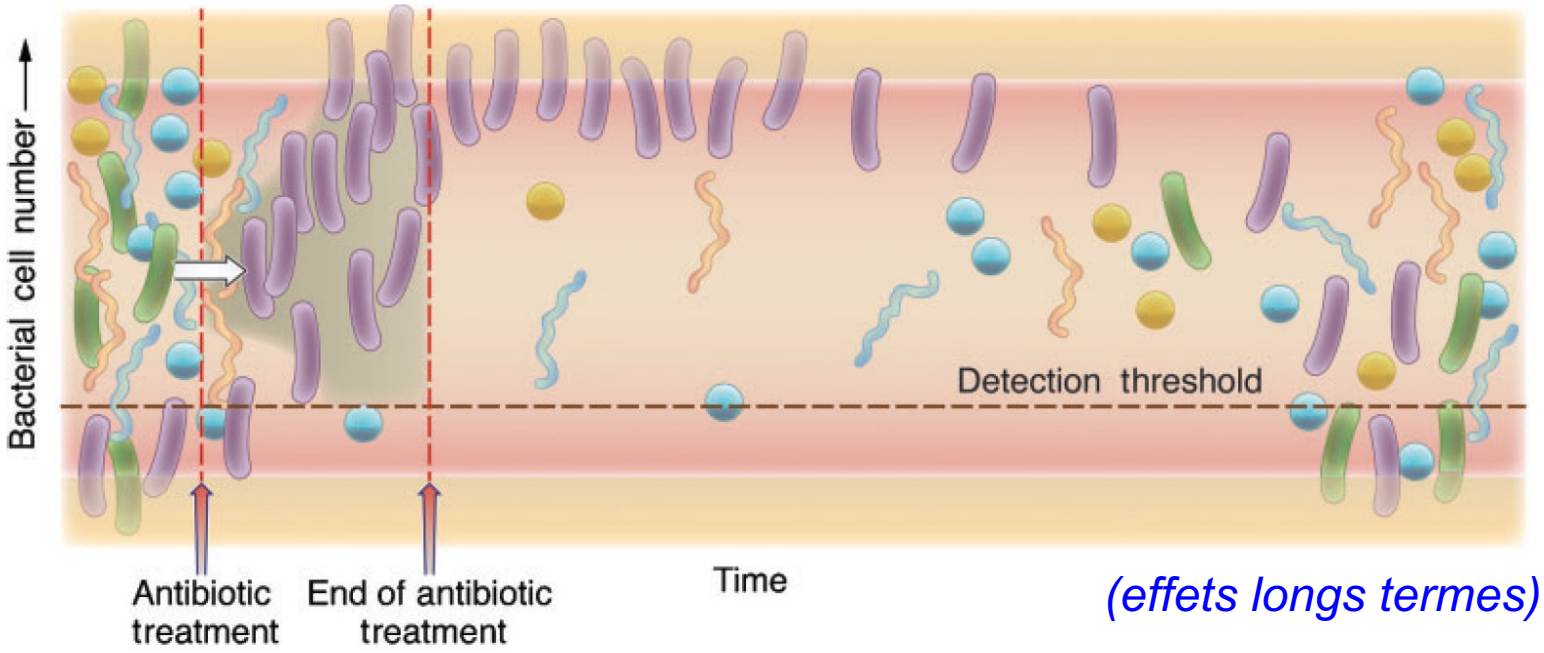
➡ **What about other MGEs ? other Ab ? active concentrations?**

➡ **Need for an exhaustive screening method.**

Effet des Ab sur la mobilité de l'ICE Tn*916* d'*Enterococcus faecalis*



Impact de l'antibiothérapie sur les communautés microbiennes intestinales (colon)



(effets longs termes)

- perte de diversité transitoire
- augmentation du nombre de bactéries Ab^R
- augmentation de la susceptibilité à la colonisation
- variable selon l'Ab, selon individu (!), selon diète, selon stress, ...

Jernberg et al (2010) Microbiology

Altération de l'abondance relative en gènes *erm* (Em^R) sur le long terme (traitement clindamycine)

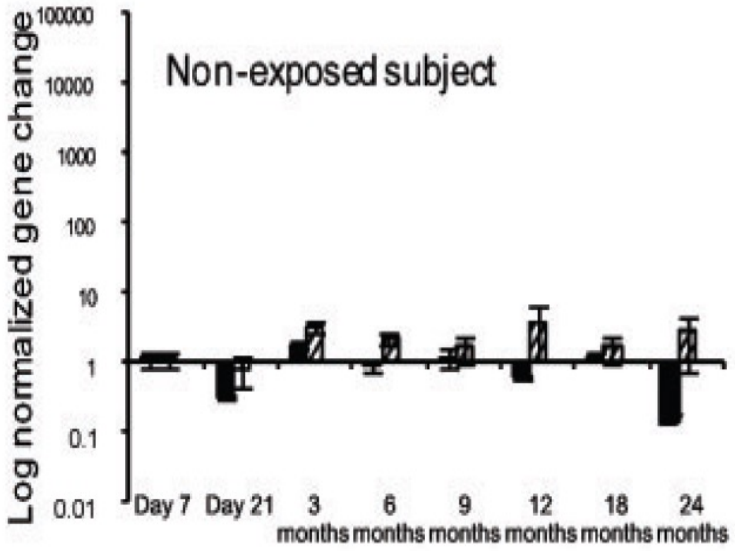
traitement clindamycine → ADN total de *feces* → qPCR spécifiques (3 gènes *erm)*

de J0 à J7

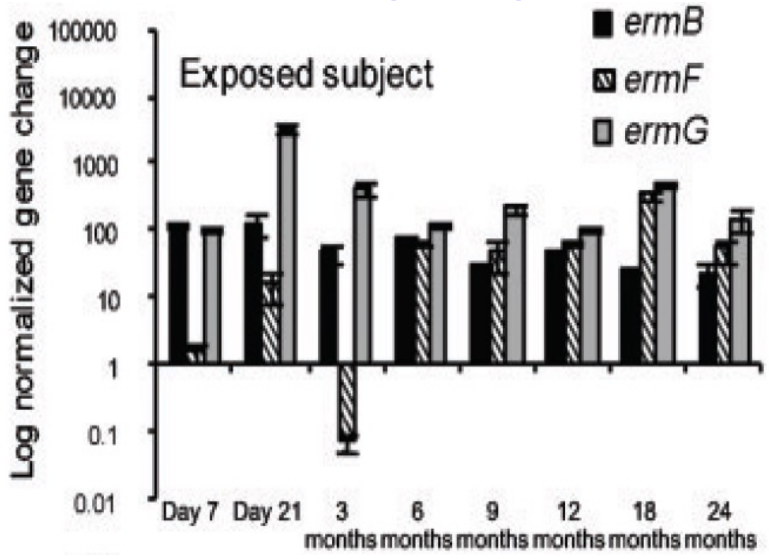
tous les 3 mois

- normalisation / ADNr 16S
- augmentation par rapport à J0

(contrôle)

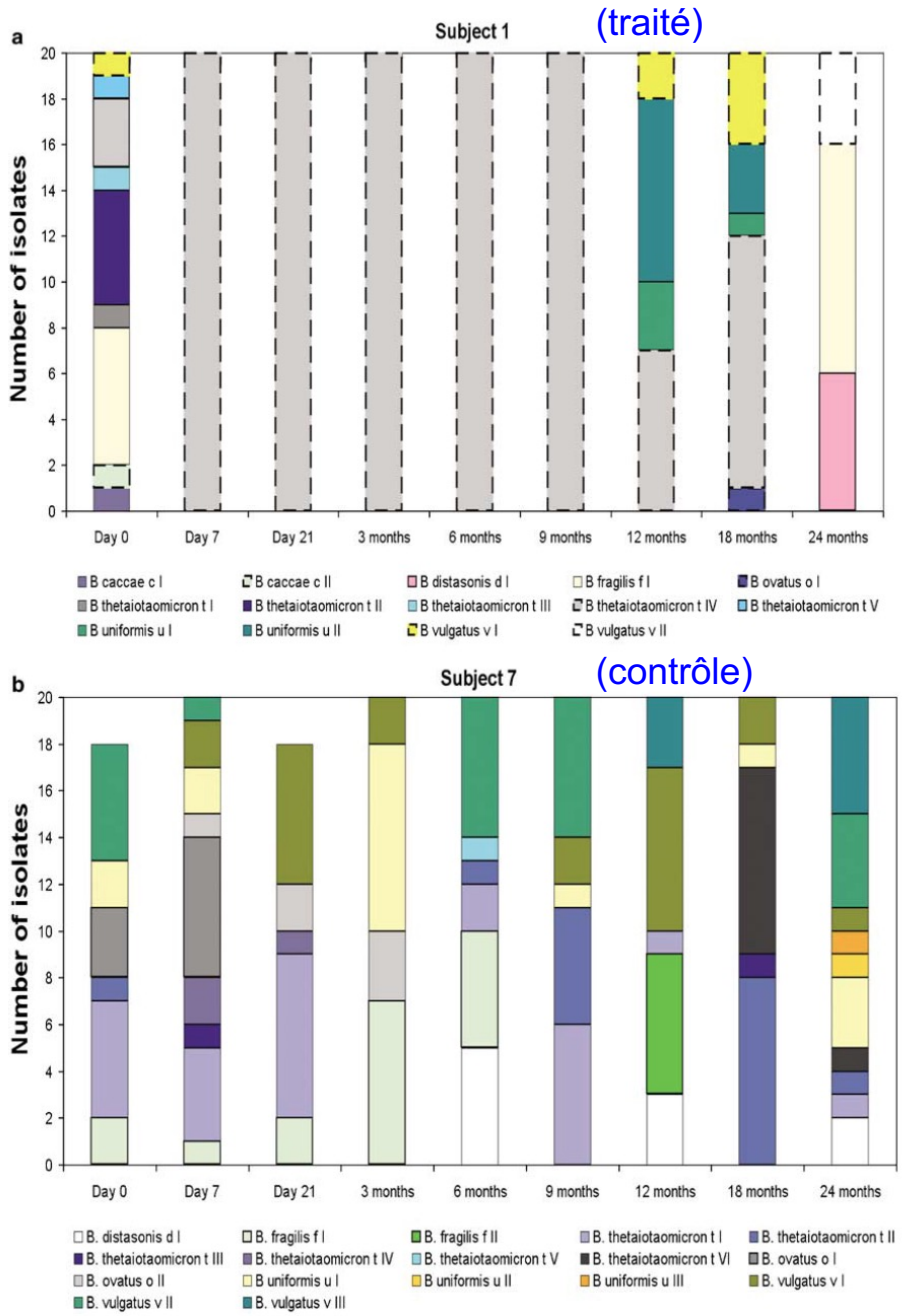


(traité)



⇒ augmentation du nombre de gènes *erm* visibles après 2 ans!
⇒ persistance de *Bacteroides* Clindamycine^R ayant acquis des gènes *erm.*

Jernberg et al (2010) Microbiology



Altération des communautés de *Bacteroides* sur le long terme (traitement clindamycine)

Approche expérimentale:
- Isolats cultivables de *Bacteroides*
- Typage par REP-PCR (code couleur)
- Résistance (points tillés)

Remarques :
- Retour au nombre initial de *Bacteroides* après 21 jours mais composition d'espèces altérée deux ans après.
- Persistance de la résistance.

Jernberg et al (2007) ISME

Impact des agents antimicrobiens sur la « stabilisation » de bactéries résistantes

La dissémination de bactéries/gènes de résistance dépend de plusieurs facteurs.

Pression :

- L'utilisation d'antibiotiques (pression majeure).

Adaptation:

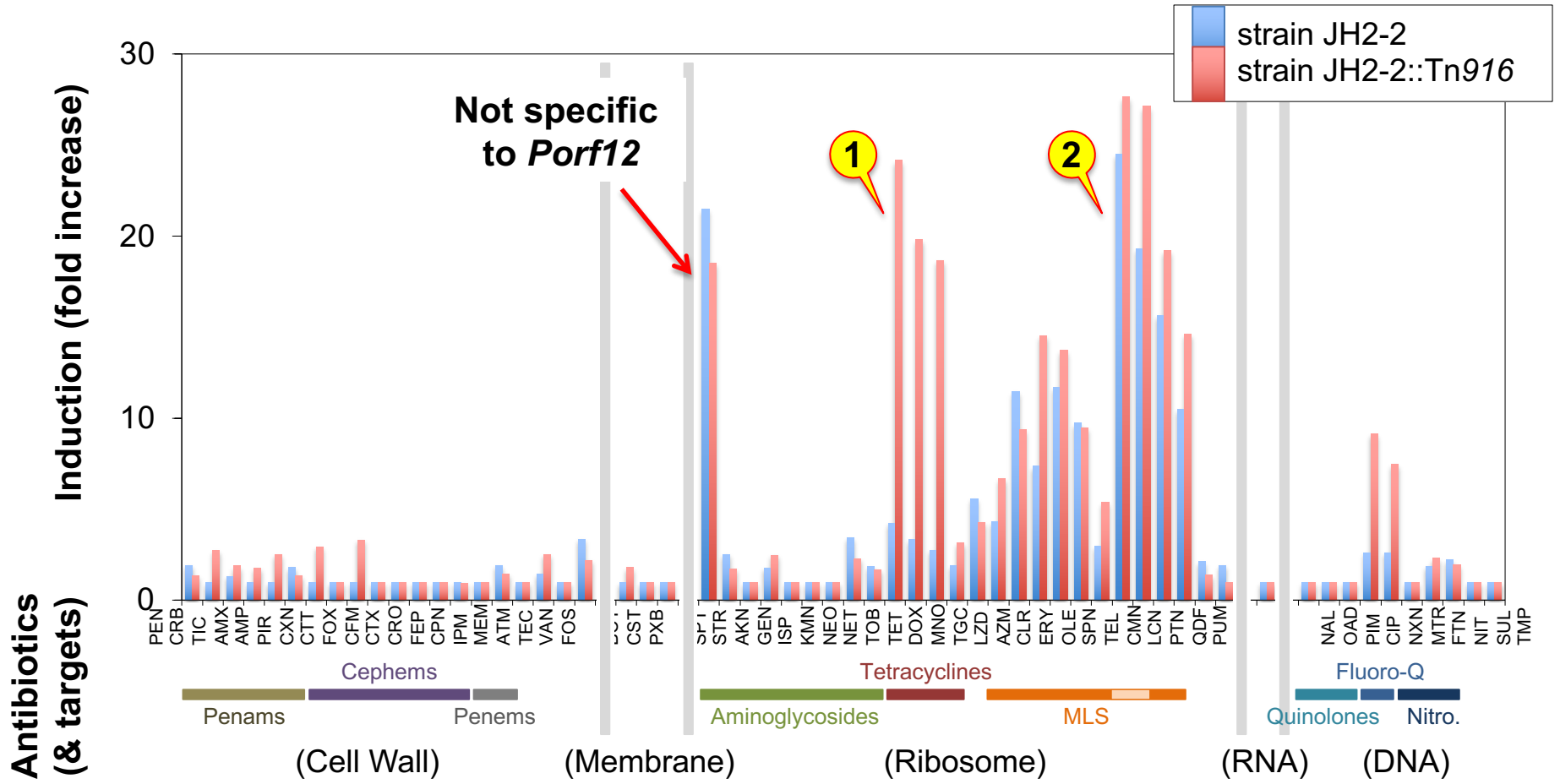
- le taux de mutation
- l'efficacité du transfert horizontal des gènes de résistance

Fitness :

- L'habilité de bactéries résistantes à coloniser l'intestin (fitness).
- Leur aptitude relative (compétition) à coloniser la niche écologique.

Jernberg et al (2010) Microbiology

Inducibility of Tn*916* mobility function by various antibiotics



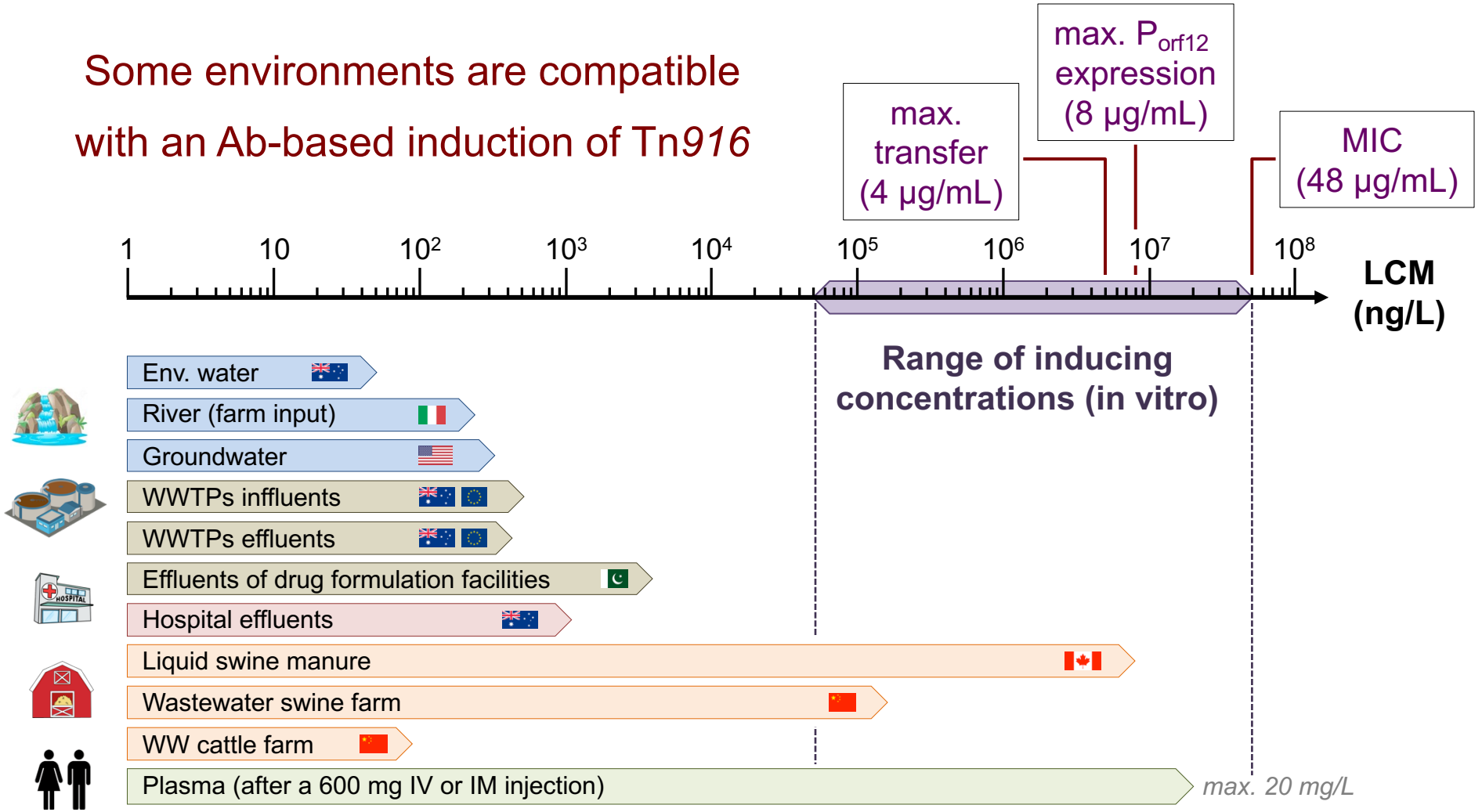
➡ P_{orf12} regulated by Ab other than tetracyclines

➡ What are the active Ab concentrations ?

Lincomycin concentrations in the environment

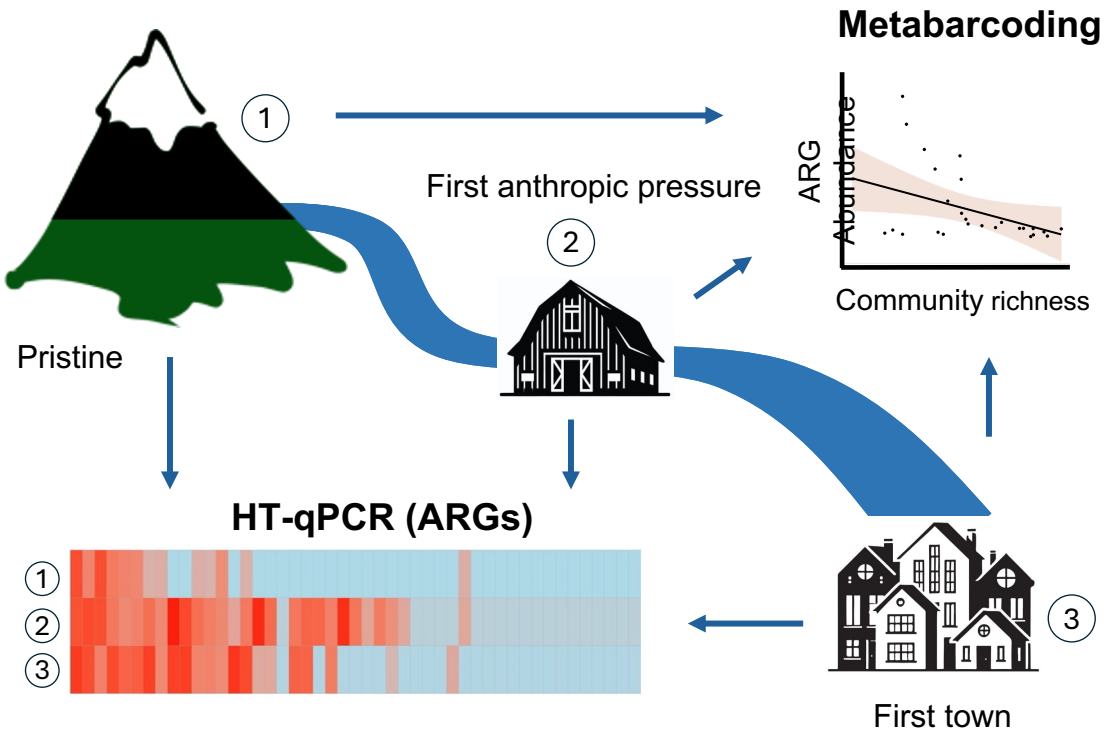
(worst case scenarios)

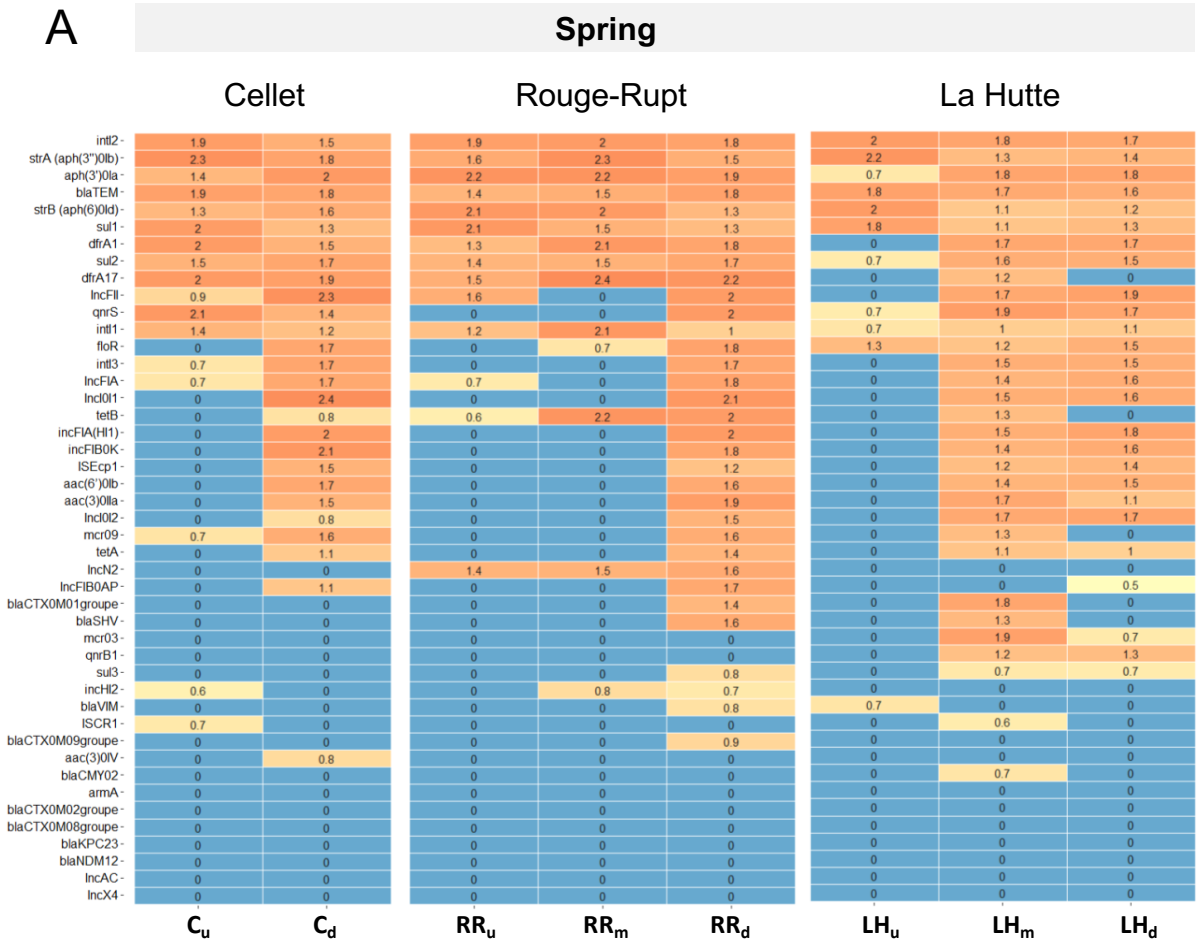
Some environments are compatible with an Ab-based induction of Tn*916*



Watkinson et al. 2009; Looset al. 2013; Barnes et al. 2008; Calamari et al. 2003; Khan et al. 2017; Kuchta et al. 2009, Zhou et al. 2013

Antibiorésistance dans l'environnement





Invasion et biodiversité (biofilms de rivière)

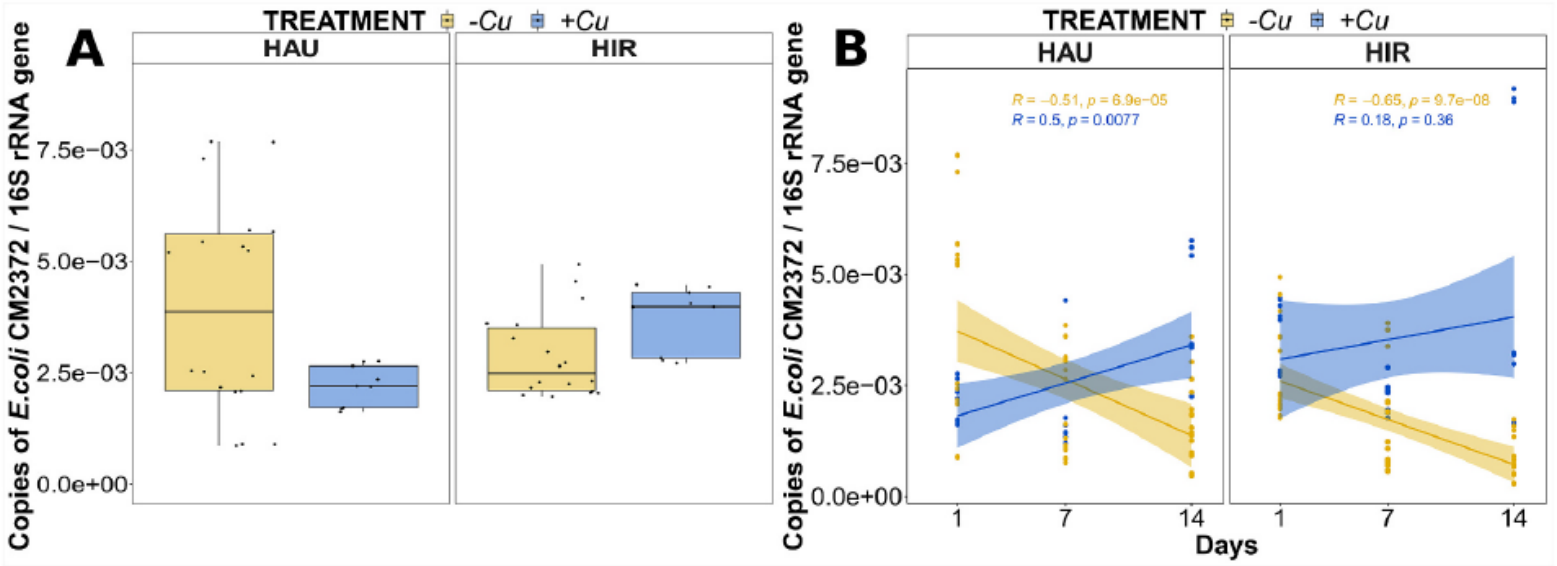
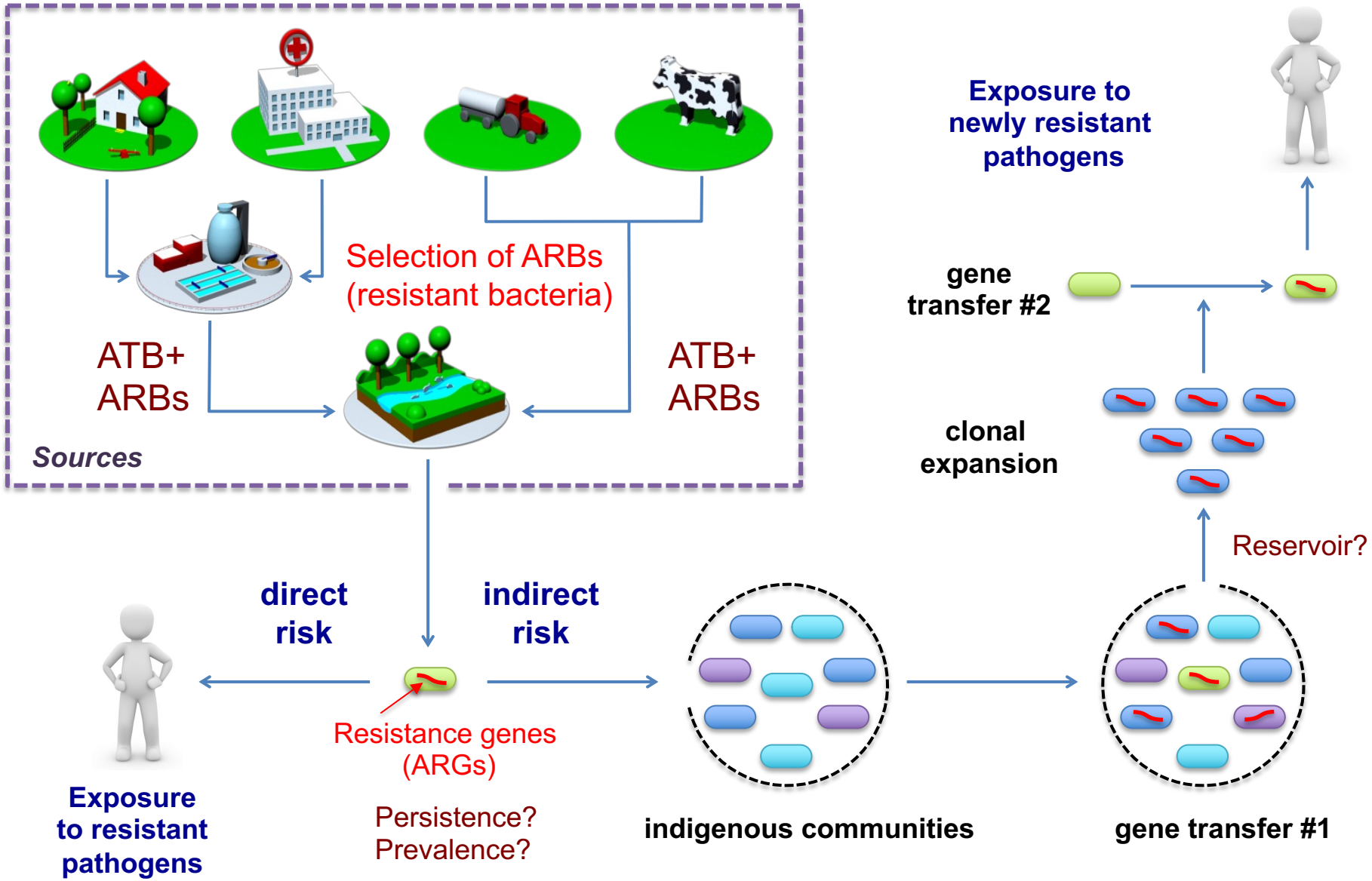


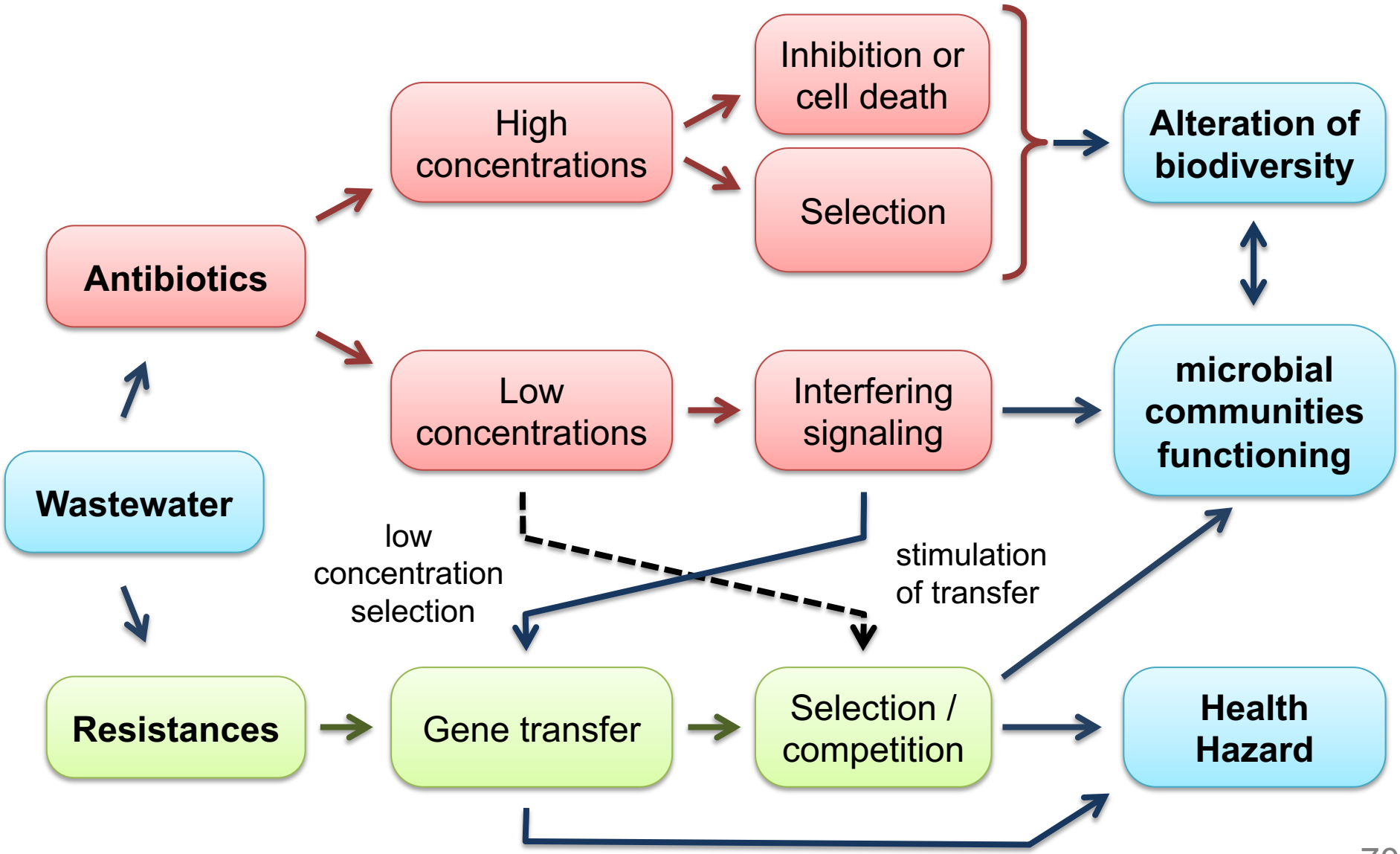
Fig. 3. Invasion and establishment of *E. coli* CM2372 into the river microbial biofilm communities. The relative abundance of *E. coli* in log scale for both river microbial communities from Hausdorfer Bach (HAU) and Hirschbach (HIR) with (+Cu) or without copper (—Cu). (A) Relative *E. coli* CM2372 abundance in log scale for day 1, the day after inoculation of *E. coli* strain representing the initial introduction into the biofilms (n = 6 biological × 3 technical replicates). (B) Linear regression for relative *E. coli* CM2372 abundance over time in the biofilm (n = 6).

Conclusion

Antibiotics and Antibiotic resistances as pollutant of emerging concern



Speculation on antibiotics' possible effects



The end

« Sola dosis facit venenum »

("The dose alone makes the poison")

Paracelse (1493-1541)

... yes but ...

Philippus Aureolus Theophrastus Bombast von Hohenheim