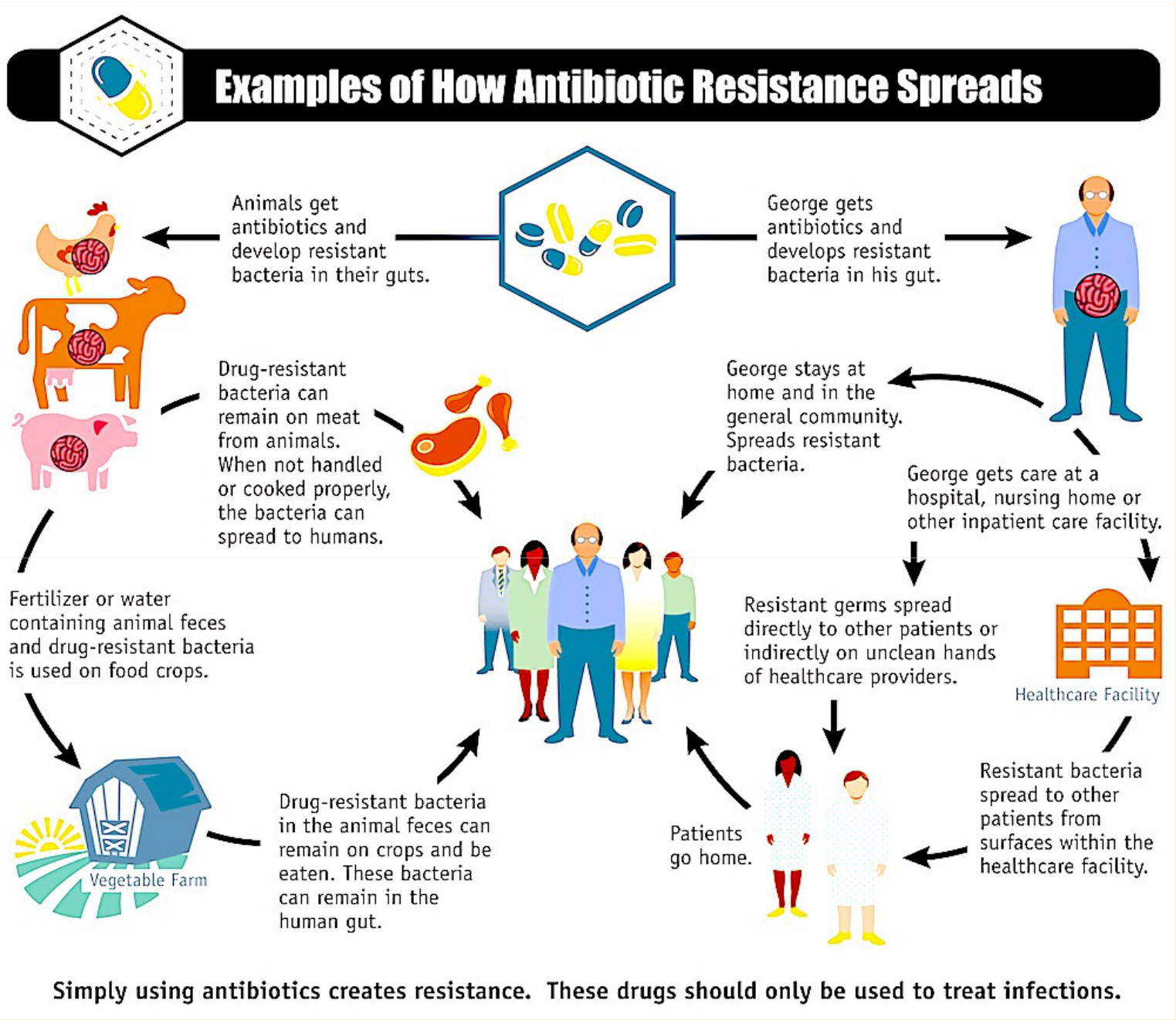
C. 2-3. / S.II.

Romeo T. Cristina®

Antimicrobial resistance and prudent use of antimicrobials in veterinary medicine

- **Antimicrobial resistance (AMR)** is a major problem, with broad implications, and is considered to be a phenomenon with a **zoonotic risk.**
- In a world where **the need for animal protein has grown immensely** due to increased population logarithmic, logically and annuity, the livestock industry benefited from the massive use of antibiotics.

- **Animal production practices** have evolved over the years to solve this population's **need for dietary protein**.
- Thus, **farms have become very large complexes** and have intensively used modern production practices **to push the growth rates of farm animals to their maximum level.**

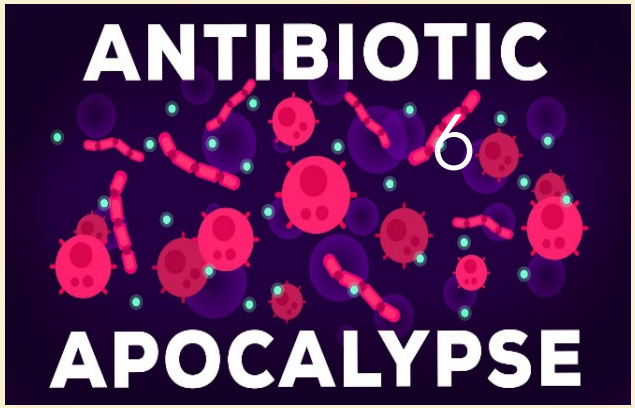


A. Ways of installing resistance to antimicrobials

Introduction

- **Antimicrobials have revolutionized medical practice,** so diseases that were once deadly, have become common and can be treated promptly.
- **This advantage** is currently **at risk, especially due to the excessive or inadequate use of antimicrobials,** which has led to increased cases of emergence and spread **of multidrug-resistant bacteria.**

According to HEINEMMANN (1999), antimicrobial agents are nearing the end of their effectiveness!



- Resistant germs **are/or become "indifferent"** ("tolerant") to antibiotics, avoiding by **various ways the expected antibacterial effect**, after the administration of usual, non-toxic doses.
- Exposure to an antibiotic = **bacteria is selected for resistance = survival of the most resistant strains** (FEDESA, 2000).
- Although **the evolution of resistance to these drugs has been predicted, the mechanisms by which the genes conferring resistance will spread have not been intuited.**

- **AMR** is a consequence **of evolution, natural selection, and genetic mutation,** this mutation being subsequently transmitted conferring resistance.
- Resistance has become **a global problem**, the main objective elements that **stimulated RAM** being:

- changes in animal production systems following the increased demand for food
- changing trends in animal trade
- increased circulation of animals and animal products
- lack of initiative and coherence worldwide

- Also, **bad practices and mismanagement** exacerbated the situation today (WHO, 2002).
- In the last decades, **resistance to anti-infectious**, antibiotic and antifungal products **has occupied dominant positions in public health concerns on the European agenda**.
- Antibioresistance **was reported a few years after**, starting in the 1950s, antibiotics began to be used in the prophylaxis and therapy of infectious diseases in farm animals.
- This phenomenon has been **progressively amplified**, as a result of the use (first of all tetracyclines, but not only), **as growth promoters in chicken and pigs**.

- An **essential fact** is that **animals can serve as mediators, reservoirs, and disseminators** of resistant bacterial strains and/or of resistance genes.

- **The reckless use** of antimicrobials in animals along with **bad practices** can ultimately lead to:
  Increased morbidity and mortality

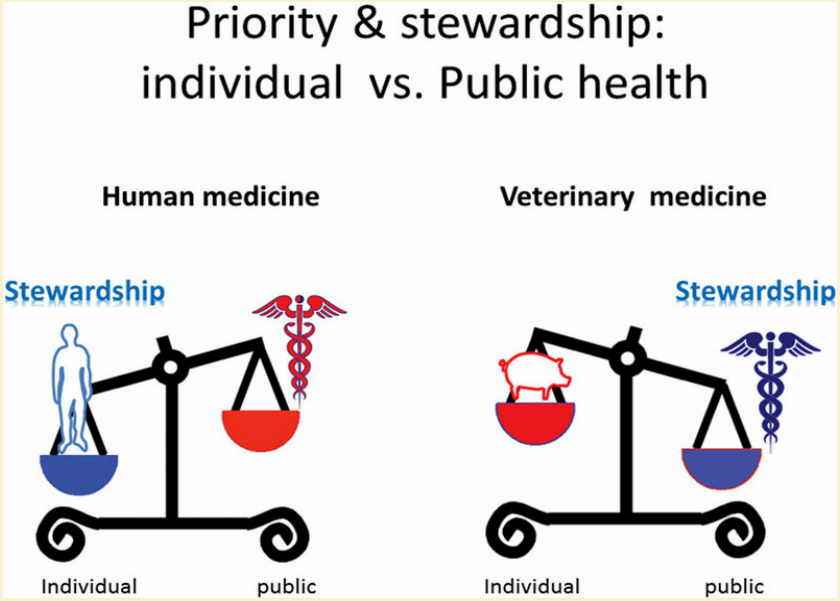
- **The reduced efficacy** of antibiotics leads to **increased healthcare costs** and **greatly increased the potential for the transport and dissemination of pathogens** with the emergence facilitated by pathogens of resistance.

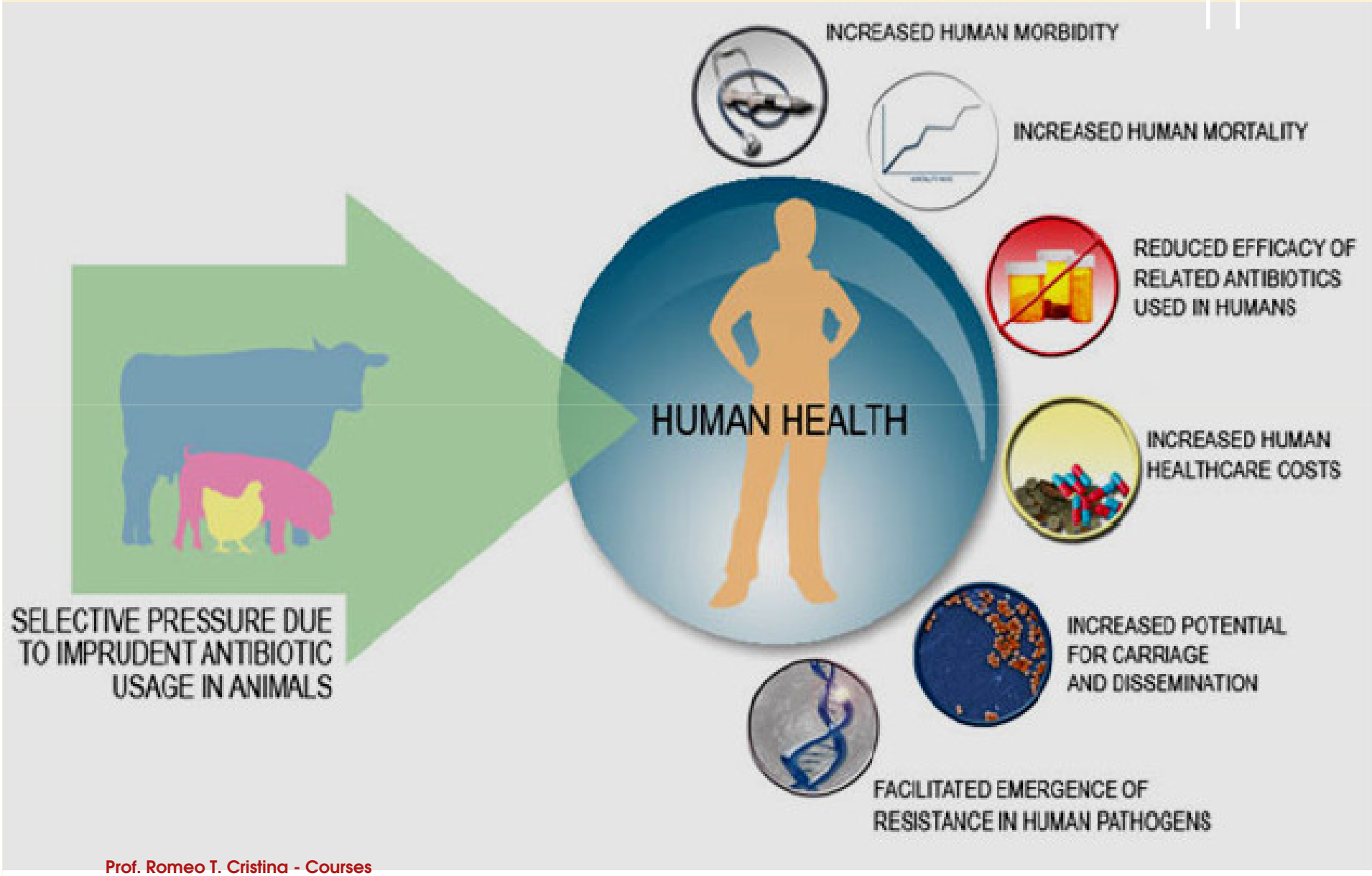
- **The spread and transmission** of resistance genes are **cross-linked** and has already been shown to be **possible between:**

  - **humans – animals**
  - **animals – humans**
  - **animals – environment**

- In this regard, it is essential to note that:

In keeping with *One Health*, sustainable veterinary antimicrobial treatment should be **linked to public health issues and not animal health issues!**



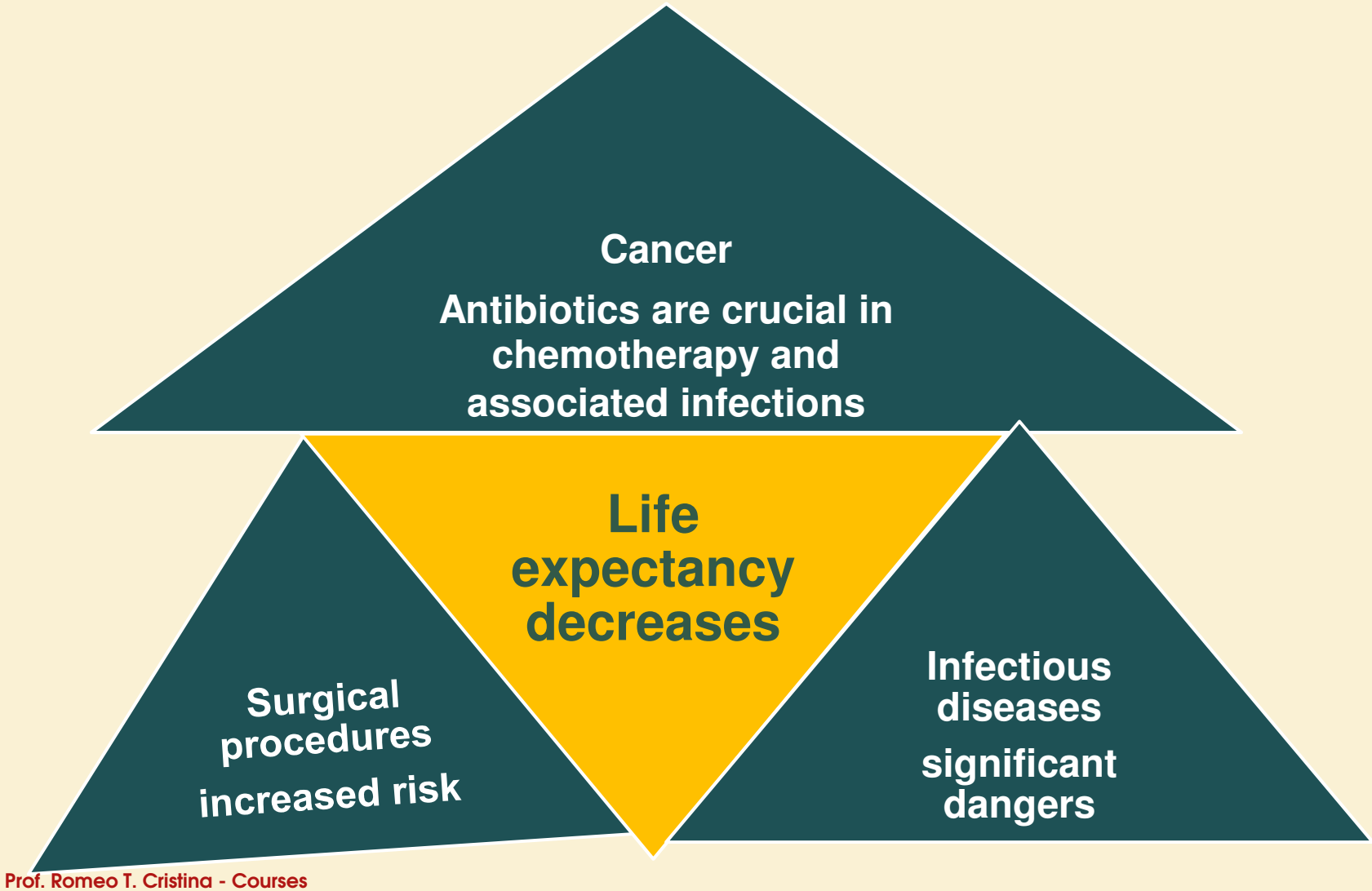

INCREASED HUMAN MORBIDITY
INCREASED HUMAN MORTALITY
REDUCED EFFICACY OF RELATED ANTIBIOTICS USED IN HUMANS
HUMAN HEALTH
INCREASED HUMAN HEALTHCARE COSTS
SELECTIVE PRESSURE DUE TO IMPRUDENT ANTIBIOTIC USAGE IN ANIMALS
INCREASED POTENTIAL FOR CARRIAGE AND DISSEMINATION
FACILITATED EMERGENCE OF RESISTANCE IN HUMAN PATHOGENS

Mechanisms + Followings

- **Irresponsible** use of antibiotics in farm animals **has developed resistance to animals or people who consume meat and by-products.**

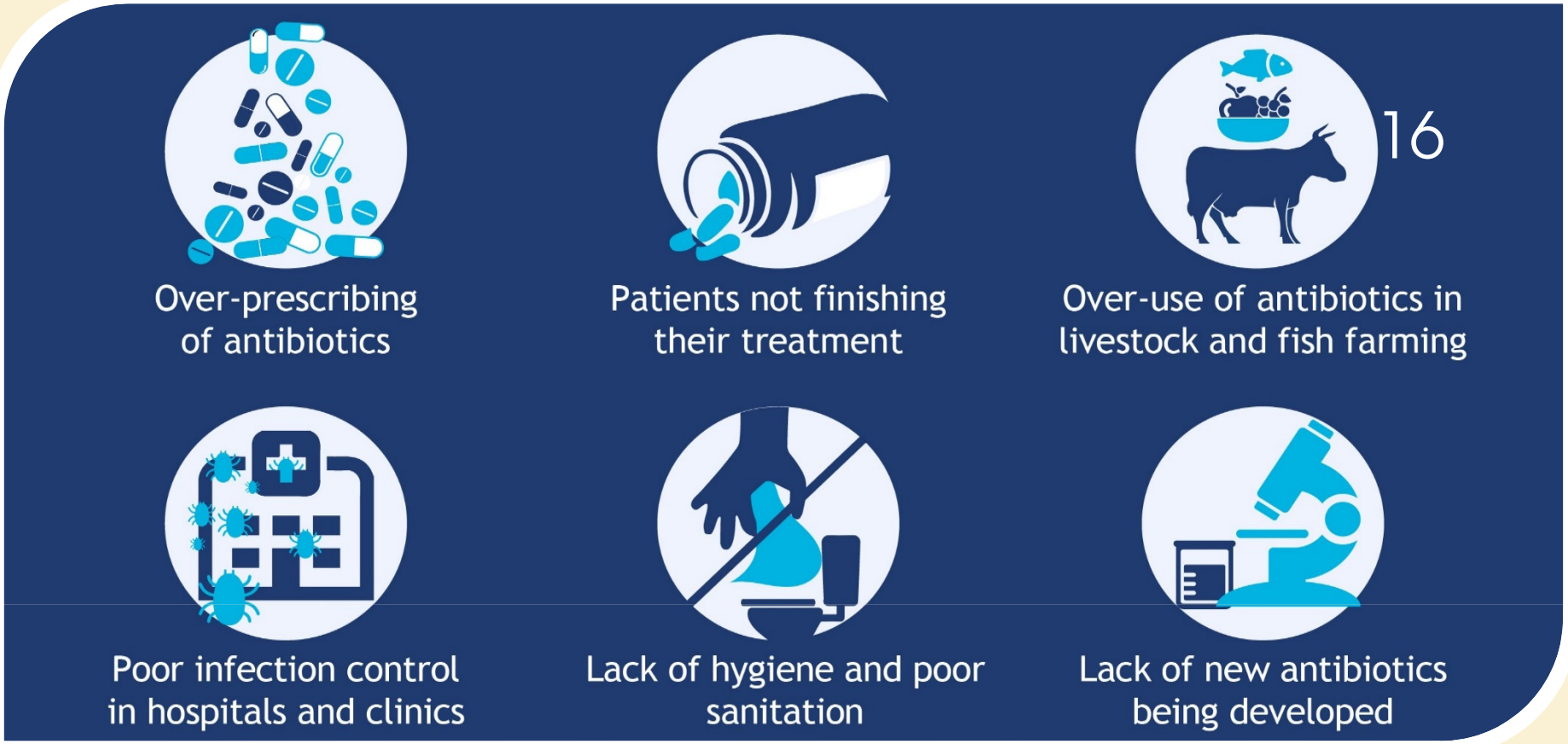
- Antibioresistance has developed mainly through the use of antibiotics as **biostimulators, in the preservation of animal foods or most often by the administration of antibiotics non-rationally, without prescription and antibiogram!**

Risks: simple infections or wounds, as well as different routine medical procedures are based on antibiotic treatment



- **Conditions** that determine the appearance of antibiotic resistance:

By selection, the bacterial genome became about **1000 times smaller than the animal/human genome!**
This fact is due to **"genome rationalization".**
There is **intense competition for resources among bacteria.**
To maintain and reproduce, the bacterial genome needs: **energy + resources**
A large genome requires **more energy** to be maintained and duplicated



Most often, this selection process **is exacerbated by human factors:**

- improper use of drugs in human and veterinary medicine
- poor hygiene conditions
- current medical malpractice
- errors in the food chain

- Genomic screening techniques **have not yet been** able to deliver their promise of **"revolution"** in the discovery of new antibiotics!

- This has resulted in time to the occurrence of **"superbugs"** = bacteria resistant to most antibiotics that are used.

- Classic examples:

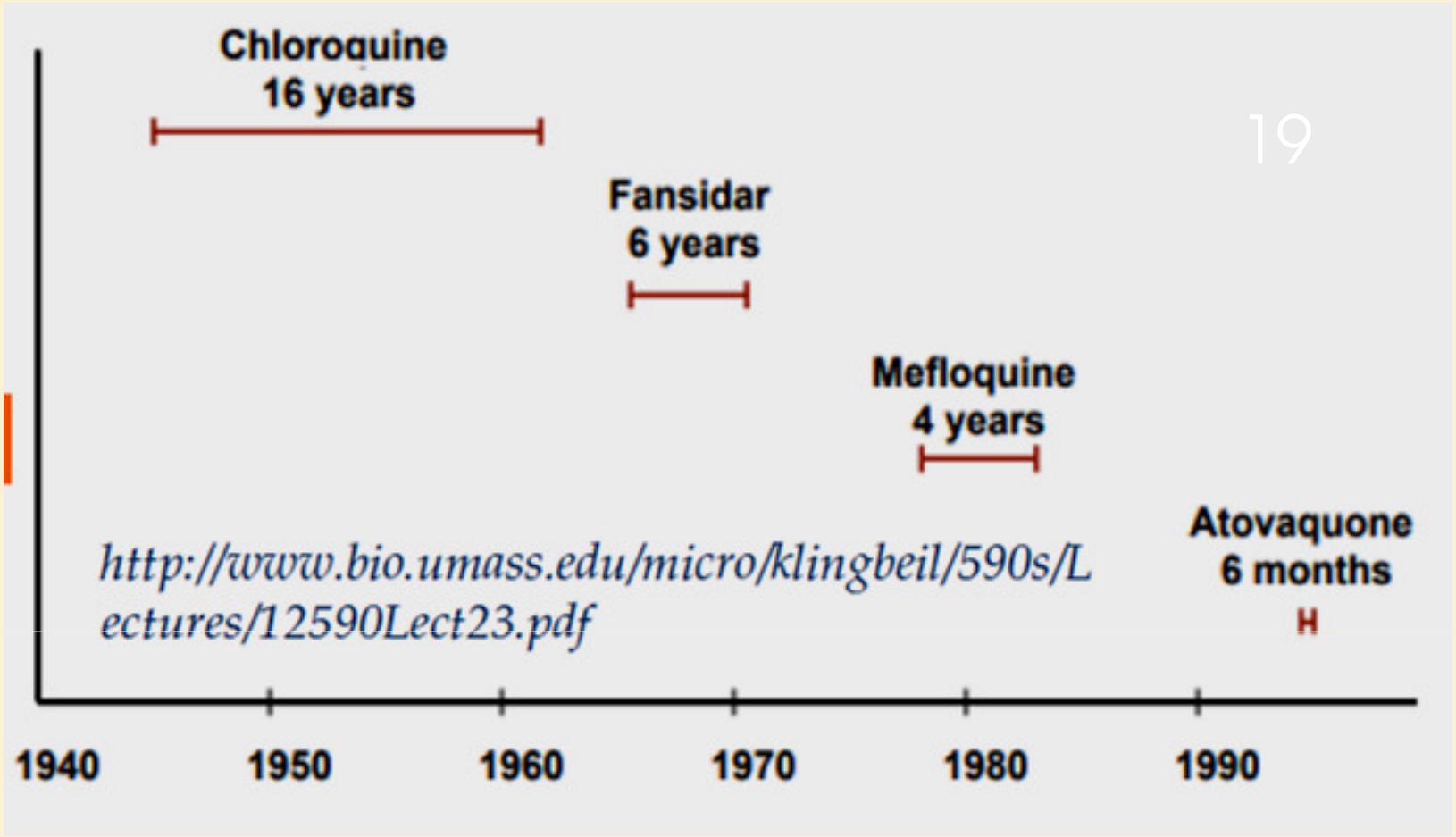
Methicillin-resistant *S. aureus* (MRSA)

***M. tuberculosis* super-resistant to antibiotics**

- **Excessive prescribing of antibiotics is not the only source of antibiotics that pollute the environment!**

- **As early as the 1970s,** antibiotics could be found in **the meat of cattle, pigs, and birds, the same antibiotics** were later identified in **municipal and groundwater systems or in the soil,** with its dramatic consequences!

For example:



The emergence of resistance to **fluoroquinolones**, after common infections with ***Campylobacter*** and ***E. coli*** in humans, is followed by their use in animal feed with the transmission of human resistant bacteria **through meat & animal products**!

Fact !

- Most reports refer to the increasing trend of the use of antimicrobial substances **in sub-therapeutic doses in cattle and birds**.
- Both molecular and epidemiological evidence indicates that the prevalence of antibiotic resistance among humans was triggered by the introduction of **enrofloxacin in poultry feed**, which prompted the FDA in 2011 **to ban the use of this drug in birds.**

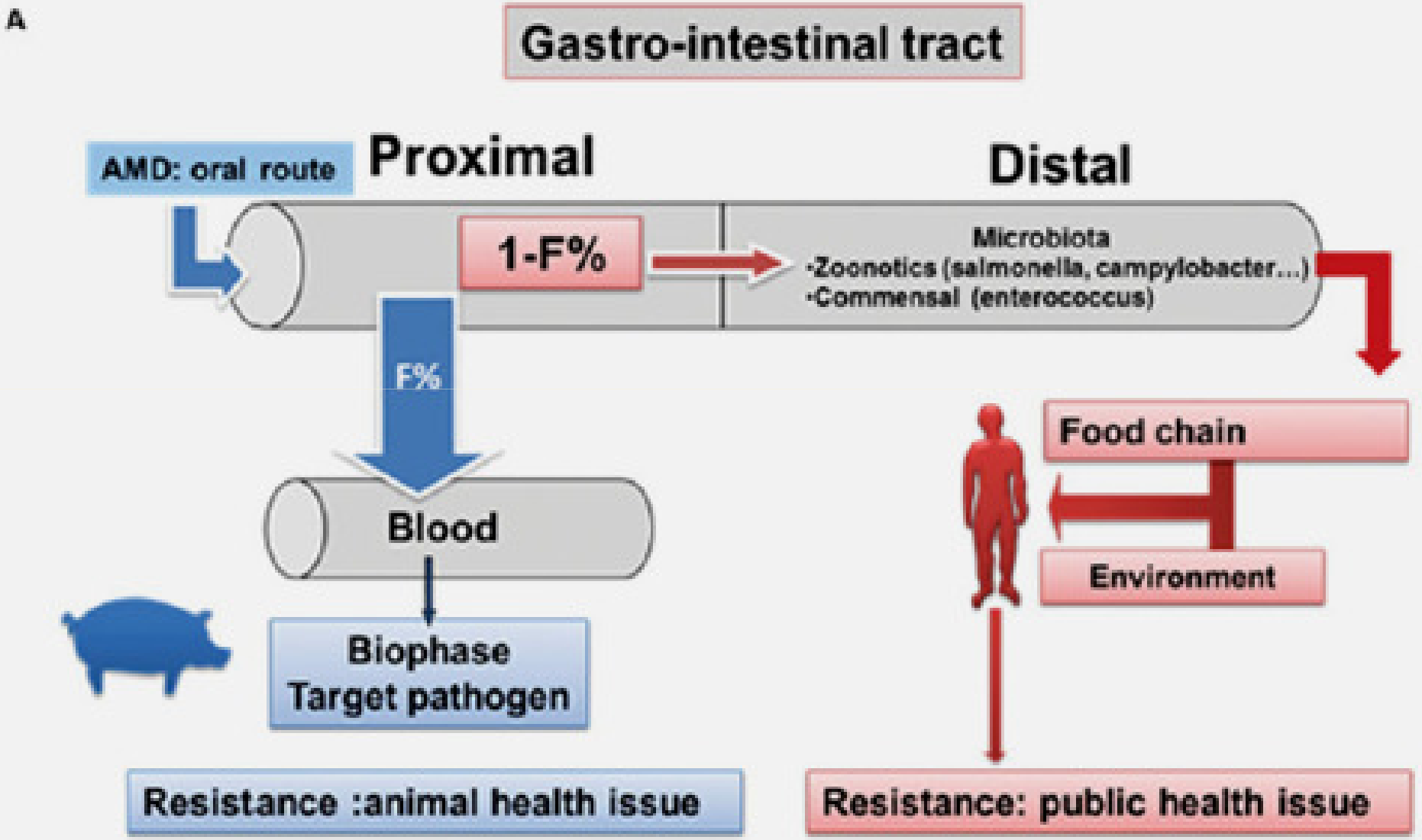
- Antibiotics given to animals **are not completely absorbed by them**!
- Depending on the antibiotic, **between 30 and 90%** of the antibiotic may be excreted in the urine or feces in a **bioactive state**, even intact or in the form of antibiotic metabolites, **which may further antimicrobial activity**.
- Antibiotics who are given to animals often reach the **soil and water** through medical waste and improperly disposed of medicines or through dust from industrial growth units.

Source:
https://www.frontiersin.org/articles/10.3389/fmicb.2016.01196/full

Impact of antibiotic administration

After oral administration

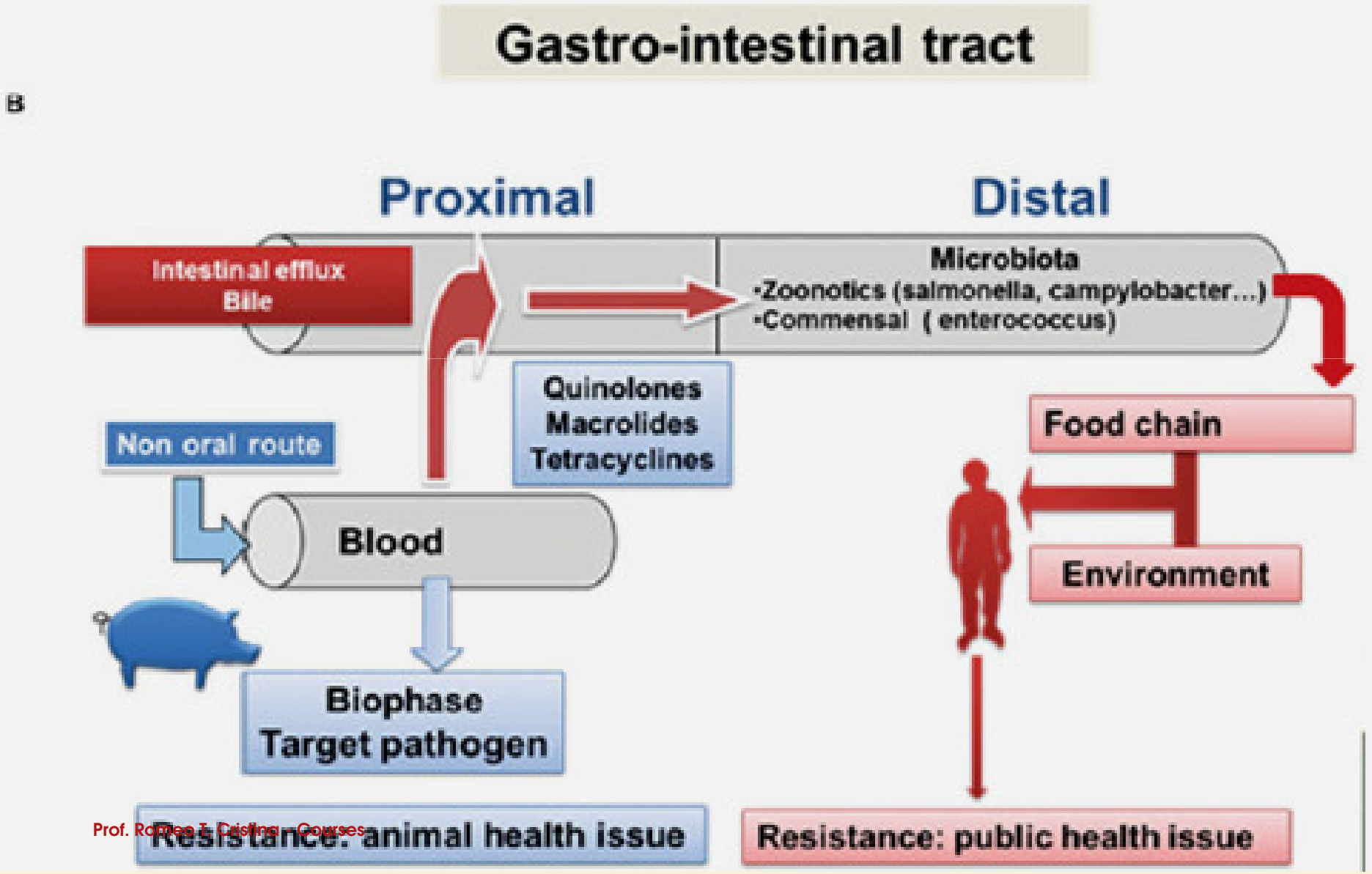
- The distal intestinal microbiota comprises zoonotic pathogens and commensal flora.
- This will be exposed to the fraction of unabsorbed drug in the proximal digestive segment = **increases local selective pressure = increases the density of bacteria and thus the frequency of resistance genes!**
- Resistant bacteria + resistance genes are released into the environment via **fecal excretion**
- These organisms and genes can reach humans through multiple pathways and eventually have access to the human GIT microbiota via the **main metabolic pathway = the food chain.**


A
Gastro-intestinal tract
AMD: oral route
Proximal
Distal
1-F%
Microbiota
·Zoonotics (salmonella, campylobacter…)
·Commensal (enterococcus)
F%
Food chain
Blood
Environment
Biophase
Target pathogen
Resistance :animal health issue
Resistance: public health issue

After systemic administration

- Most a.u.v medicines are eliminated, and reach the GIT, either through **bile secretion or intestinal clearance** = drug **concentrations** appear capable of selecting resistant organisms = **negative effects**
- Also, the impact on the animal TGI microbiota of antibiotics is not limited to the **aerobic fraction** - approx. 1-2% of the total microbiota; (usually *Escherichia coli* and *E. faeciens* - considered sentinel bacteria) - can also have a negative impact on the **anaerobic population** (which is a much larger sequence)!

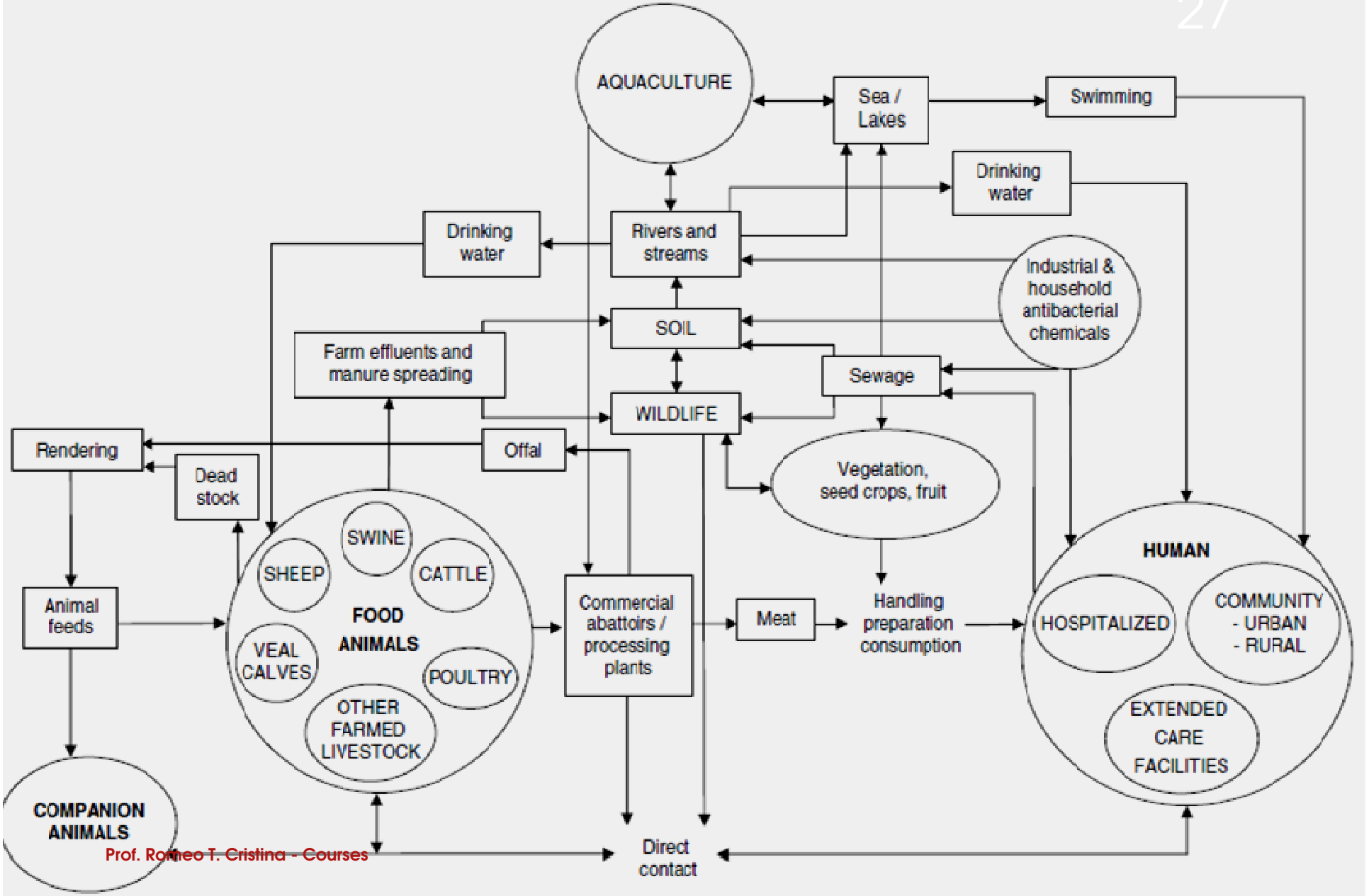
Source:
https://www.frontiersin.org/articles/10.3389/fmicb.2016.01196/full



- **Antibiotics** that have been identified in surface waters often included:

• macrolides,
• sulfonamides
• tetracyclines,
• chloramphenicol
• chlortetracycline,
• sulfamethazine
• lincomycin
• trimethoprim,
• sulfadimethoxin
• sulfamethazine

Transmission of resistance in nature



In Romania

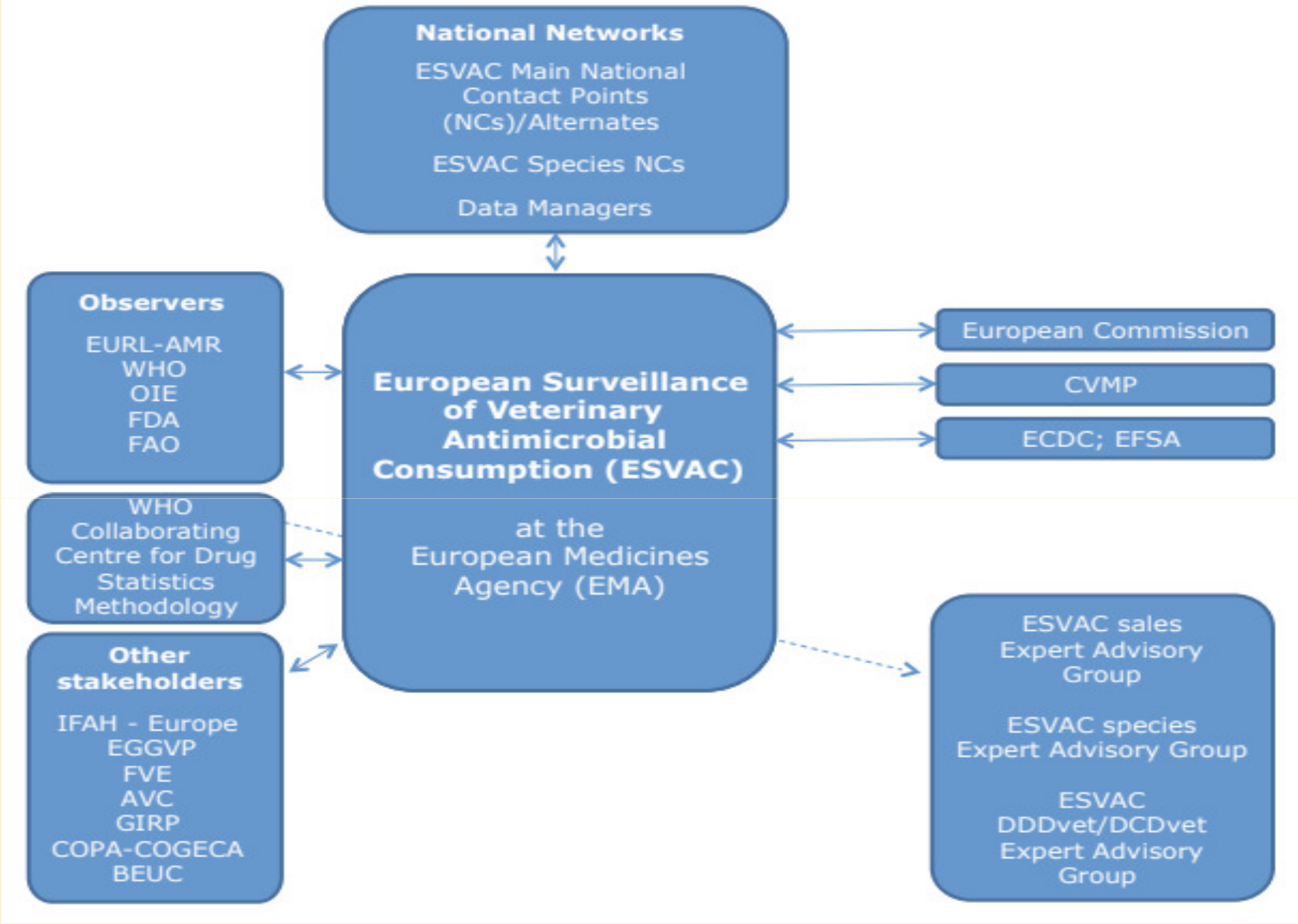
- **the main causes** that favor the emergence of phenomena of resistance to drugs are:

• inadequate **dosing/under-dosing** of active substances

• **treatment of viral diseases in animals with antibiotics**

•**wide-spectrum antibiotics administered at any treatment, while narrow-spectrum drugs would be sufficient**

- At continental level, the monitoring of the phenomenon is coordinated by the **European Center for Disease Prevention and Control (ECDC).**
- The monitoring is carried out by the **EARSS (European Antimicrobial Resistance Surveillance System)** through the **EARS-Net (European Antimicrobial Resistance Surveillance Network)** system, to which 28 countries, including our country, have joined, the first such monitoring being carried out in 2009.



Institutional interrelationships

Studying the antibiotic efficiency

The most important **tests** are:

a). The biological assays:

Allow a precise & sensitive determination of antibiotics

Diffusion method = usual technique for β-lactams

Detectable limits = order ng / mL

b). Physico-chemical assays

- Usual methods: spectrophotometry, fluorometry high-performance liquid chromatography (HPLC)
- less sensitive, vs. the biological tests = are dependent on methods of preparation and the samples' purification.
- **HPLC** = is the most versatile with perspectives of usage.

c). The In vitro tests

determine the susceptibility of a strain isolated from diseased animals in order to select the correct therapeutic mean.

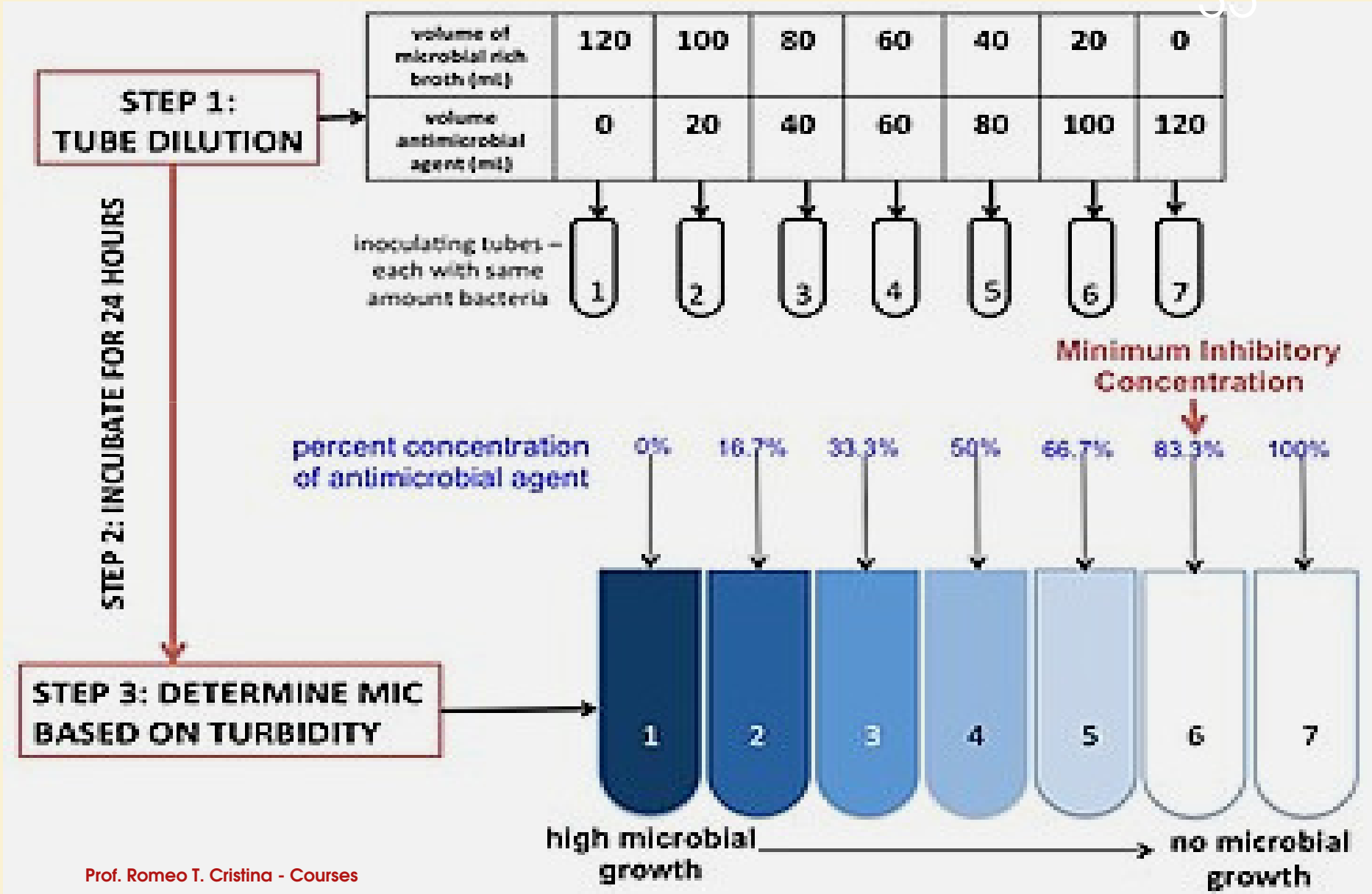
The Disc method (DM)

- Qualitative test based on the application of antibiotic impregnated paper discs placed on inoculated Petri plates.
- Disadvantage = the need to isolate pure cultures for tests = postponement of the results for 24-36 h!
- Under field conditions, time = too long, so, it is takes to much time when dealing with an animal in need of an optimal treatment.
- Resolving (commonly chosen by practitioners) = starting broad-spectrum antibiotic treatment, without waiting for the results.

The Minimum Inhibitory Concentration (MIC)

- Quantitative measure of a bacterial population to an antibiotic.
- the most common standard method of analysis of the effectiveness of an antibiotic.
- MIC = by increasing the microorganisms concentration in successive concentrations of antibiotic → the lowest concentration = MIC.
- The value obtained isn't absolute because it is affected by variables (e.g. number of organisms inoculated at the beginning of the test (which should be approximately 105 bacteria / mL).

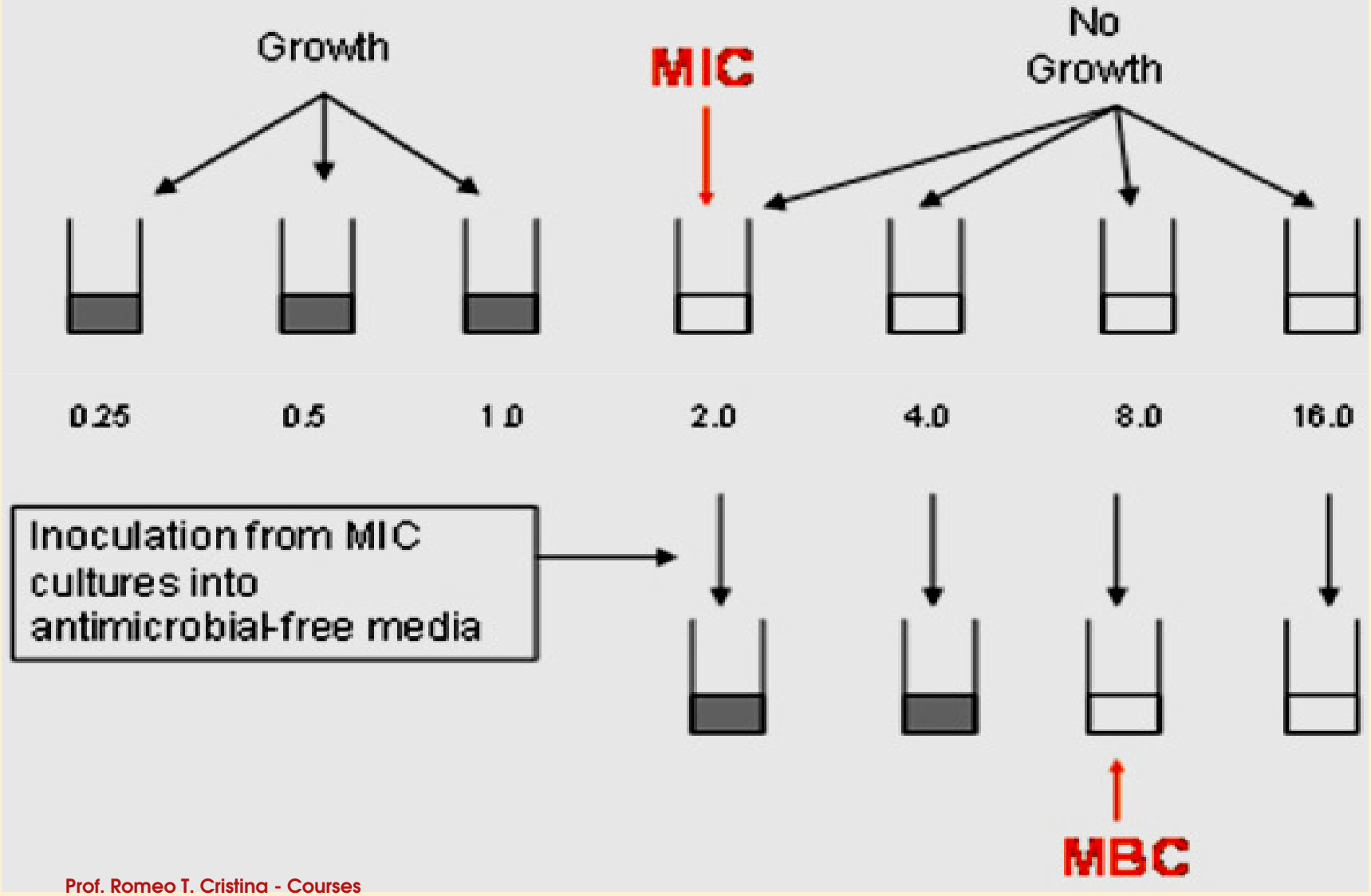
The tube method



The Minimum Bactericidal Concentration (MBC)

- **High concentrations**, that will be added to a new range of antibiotics ⟶ After incubation, the tubes are re-seeded in adequate medium and **compared** with a control subculture of tubes, obtaining three variants:
- similar growth with control tubes = **bacteriostatic**
- disparate growth = **incomplete bactericidal**
- no growth, lowest concentration = **MBC = full bactericidal**,

Serial Dilution Susceptibility Testing



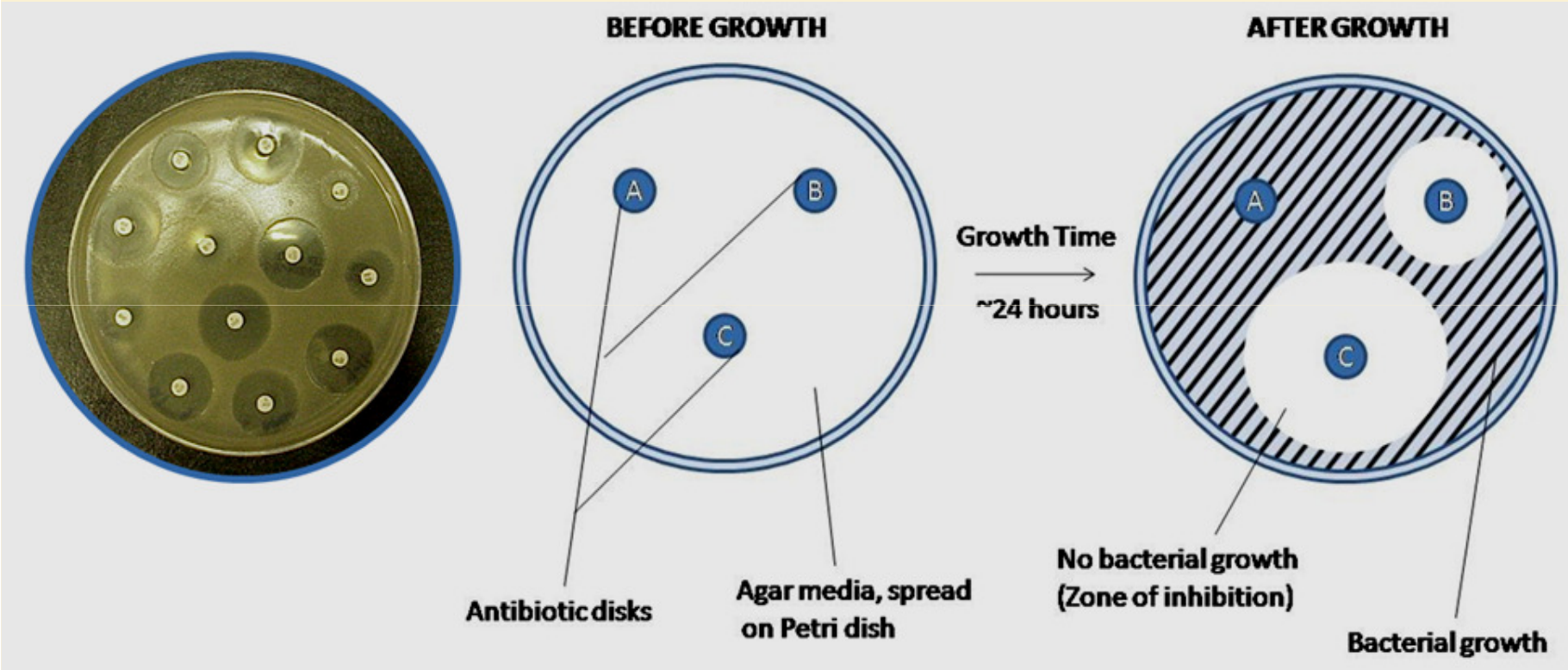
The Minimum antibiotic concentration (MAC)

- In vivo antibiotics = therapeutic activity with lower concentration than MIC, therefore, interpretations like serum concentrations, cannot be taken ad literam to express an antibiotics efficiency.

- MAC = the concentration of an antibiotic that will reduce the growth of an organism in vitro by a factor of 10 (i.e. 1 log).

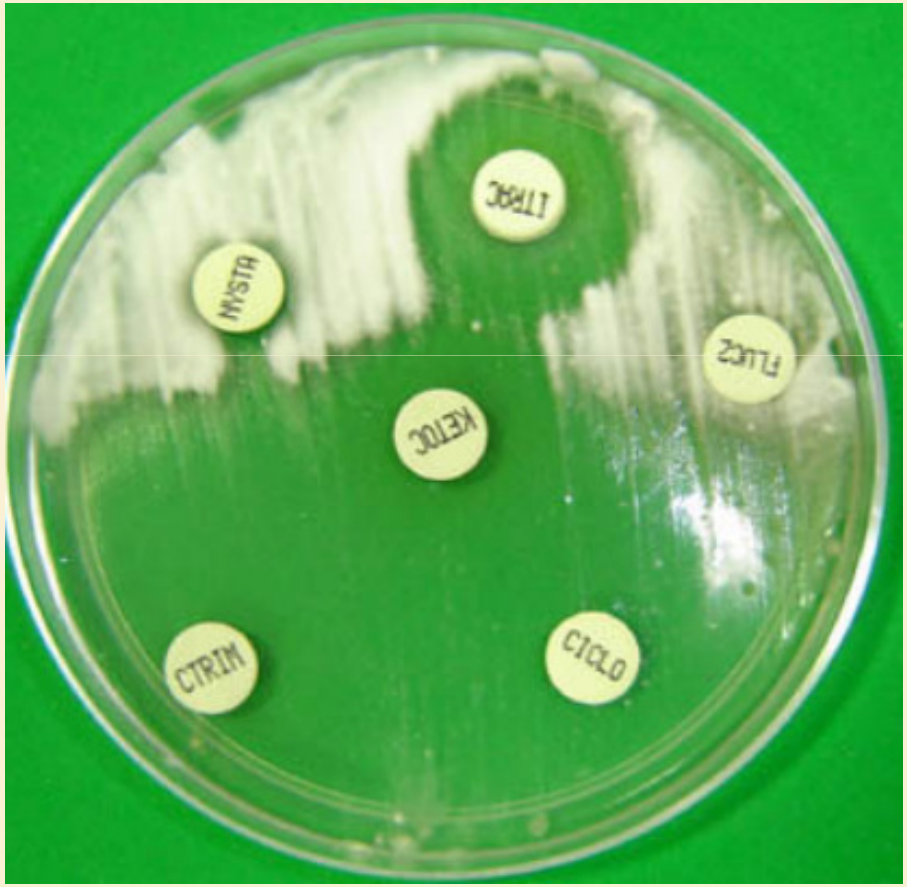
- The MAC value should be = a quarter or one-tenth of the MIC, depending on antibiotic and organism.

Antibiogram



Kirby-Bauer – The disk diffusion test

Antifungigram

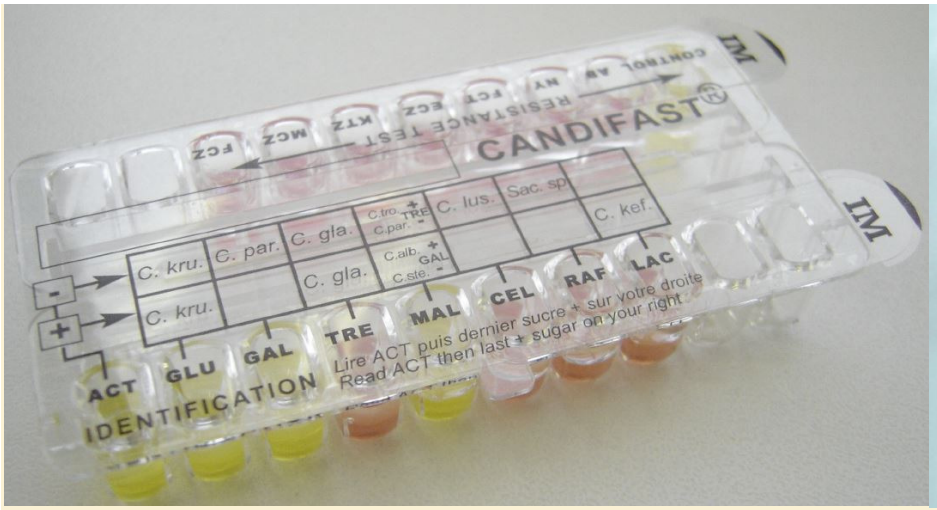


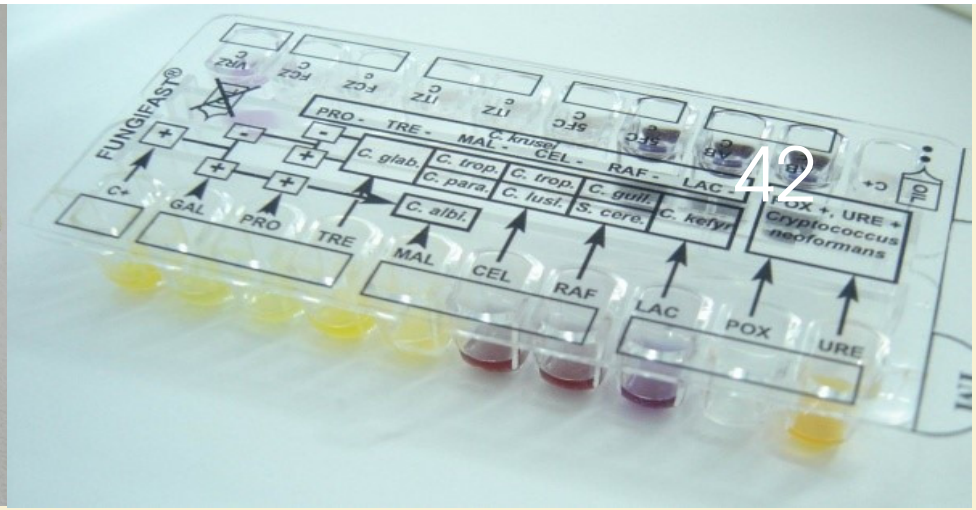
Examples of kits for the detection and determination of resistance to antifungal

FungiTest

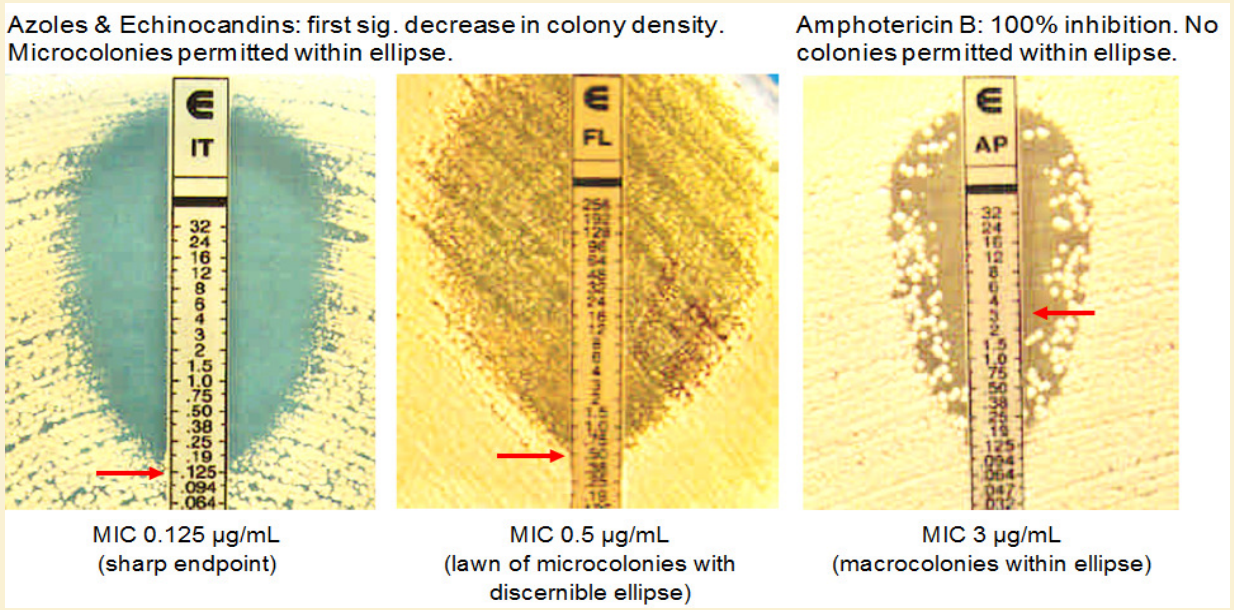
- is a kit that tests the sensitivity of the following antifungals: *amphotericin B, ketoconazole, miconazole, itraconazole, fluconazole, and flucytosine*.

- the test can specify if the strain is sensitive, intermediate sensitive or resistant by testing the sensitivity at two different concentrations for each antifungal

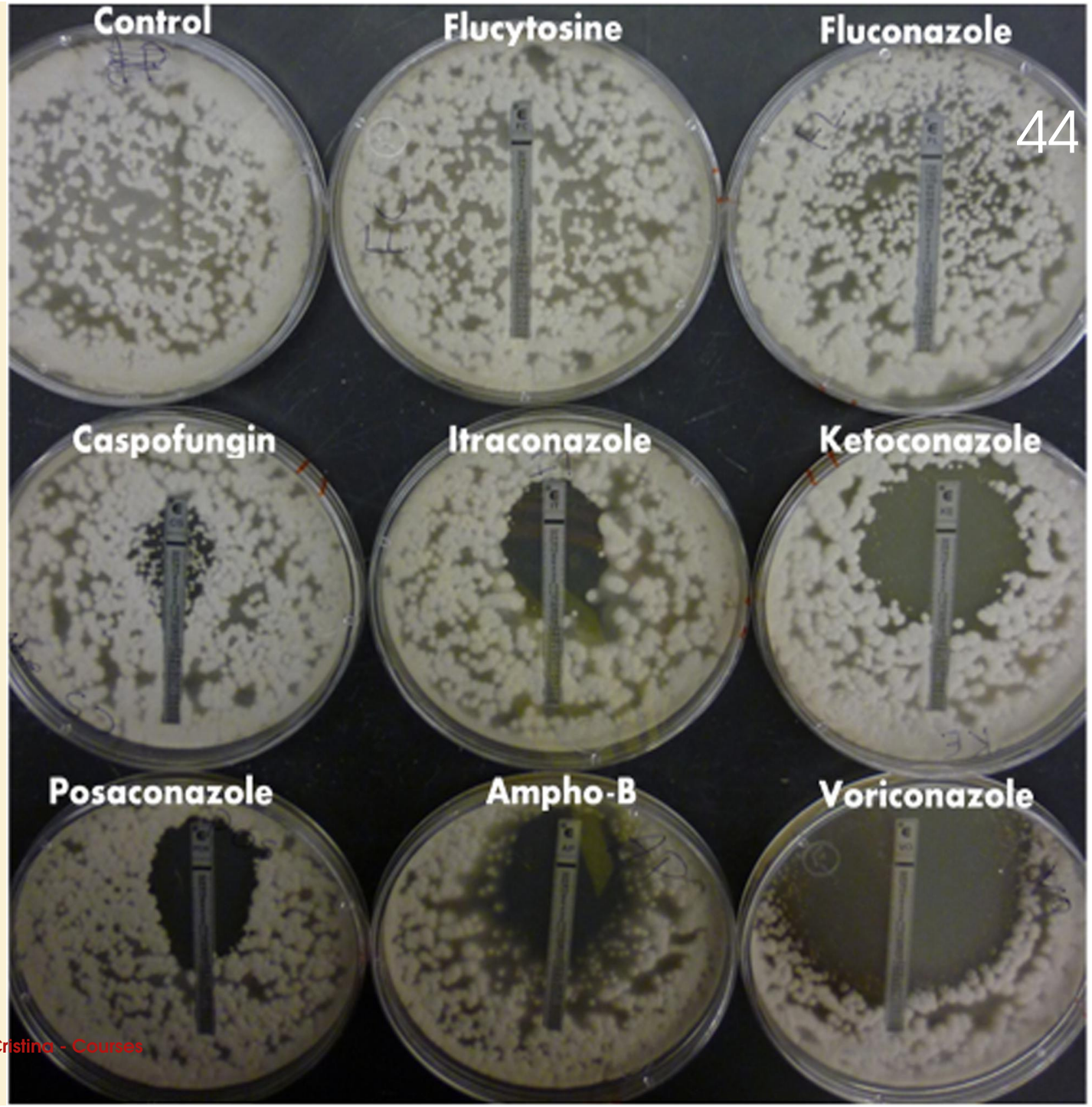




- **Candifast and Fungifast** combine the identification and testing of antifungal resistance of the main yeast pathogens. The tests are performed in galleries of 20 wells, ready for use, which is inseminated from the same inoculum.
- **Candifast** is an identification kit based on fermentation tests and determination of the presence of urease. This test can identify **8 Candida species and specify whether the strain tested is part of the genera Trichosporon, Geotrichum or Rodothorula, but cannot specify the genus or species. Candifast also contains a sensitivity test kit.**
- **Fungifast** allows the identification of the following yeasts: ***Candida albicans, C. guilliermondii, C. lusitaniae, C. parapsilosis, C. kefyr, C. krusei, C. glabrata, C. tropicalis, Cryptococcus neoformans, Saccharomyces cerevisiae***

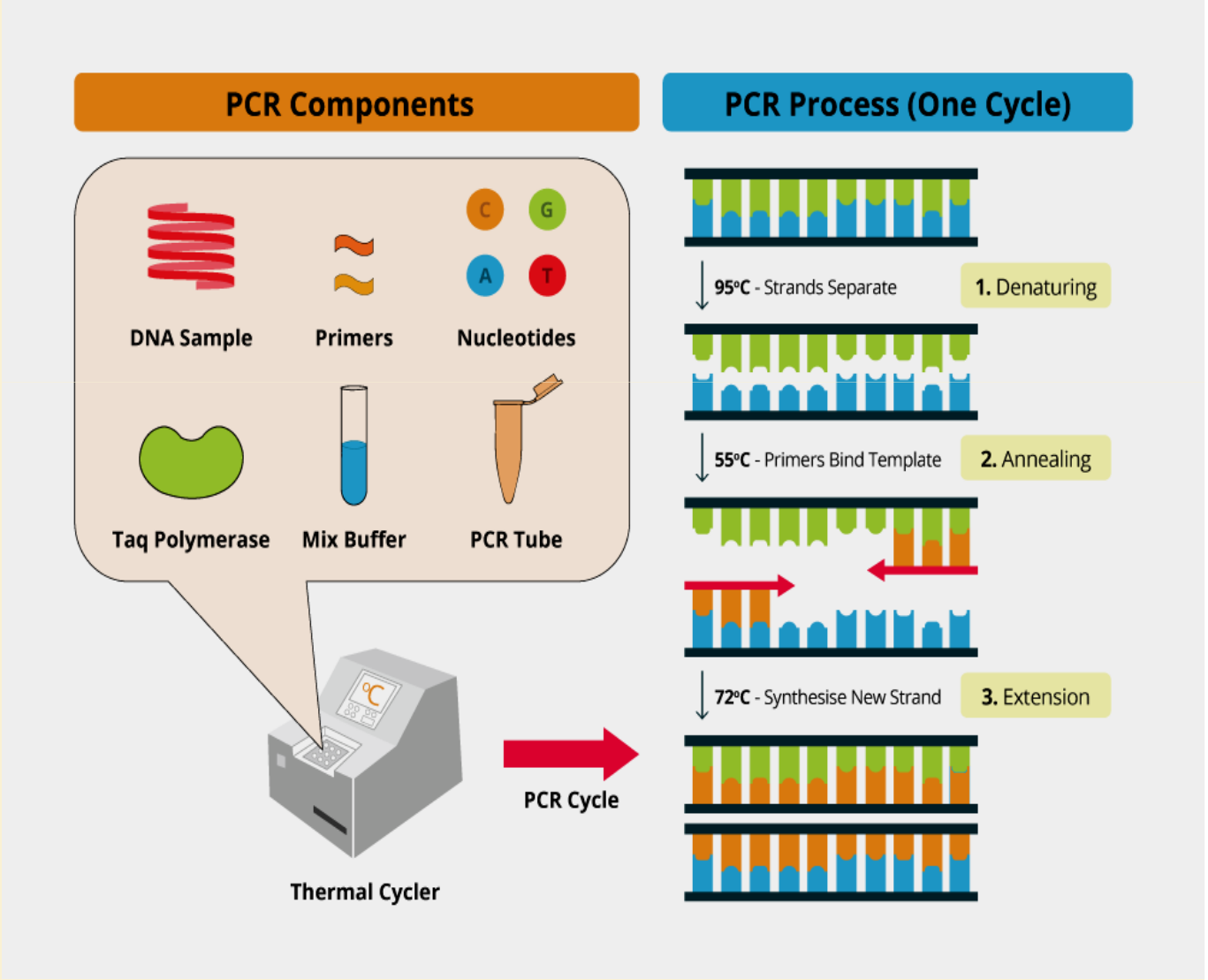


- **Etest, (known before Epsilometer test)** manufactured by bioMérieux, is a in vitro diagnostic kit used to determine MIC (minimum inhibitory concentration) and whether or not a bacterial or fungal strain is susceptible to a specific antimicrobial action.
- **Etest** - inert and non-porous strip, with a predefined antimicrobial gradient, covering a continuous concentration range, for determining the MIC values of a wide range of antimicrobial agents.

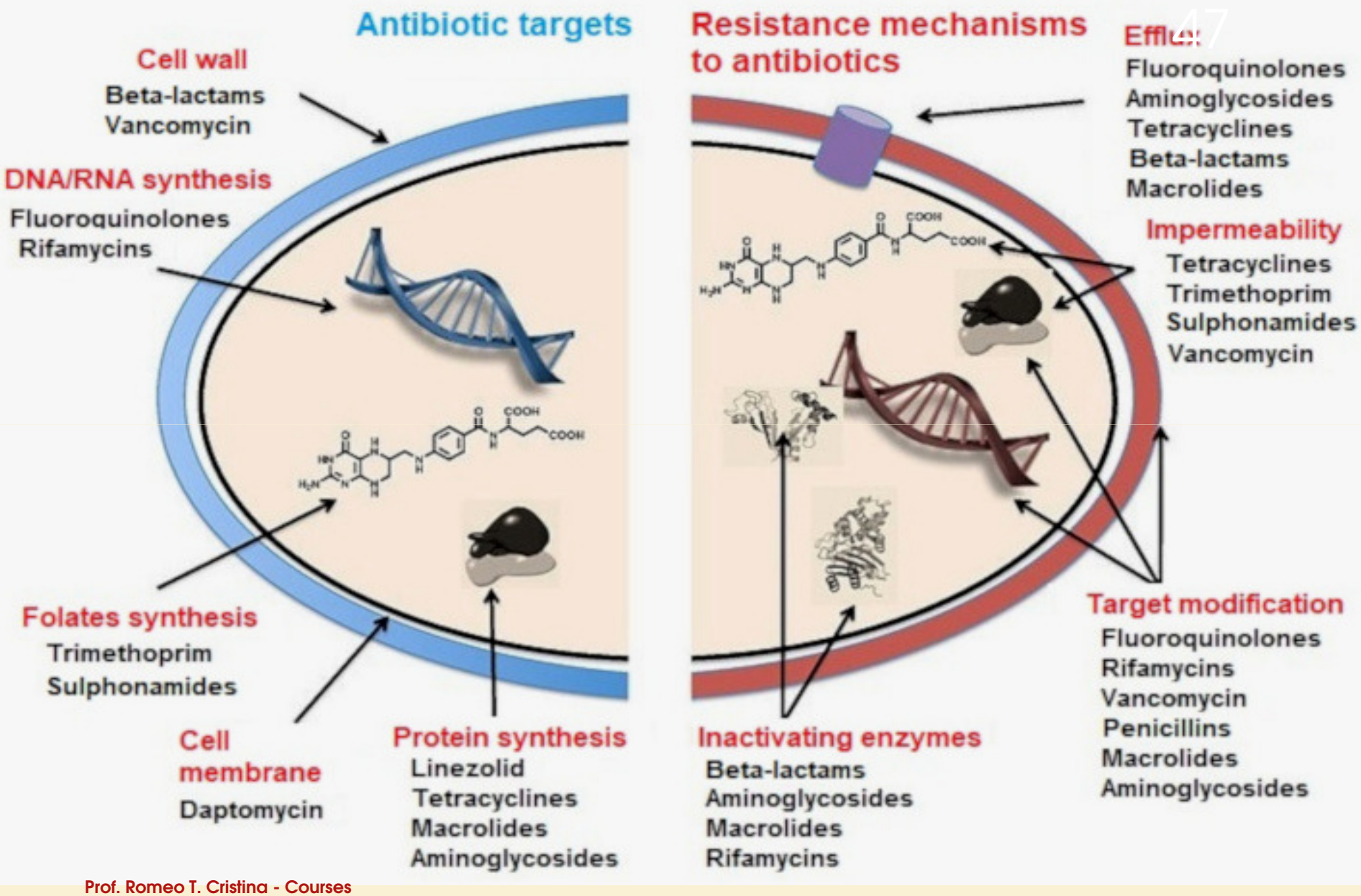

Control
Flucytosine
Fluconazole
Caspofungin
Itraconazole
Ketoconazole
Posaconazole
Ampho-B
Voriconazole

Modern identification methodologies

- for animals and humans, genes encoding antimicrobial resistance were detected by molecular biology techniques, respectively:
- • Polymerase Chain Reaction (PCR) in several variants and through
- • Real-time PCR



B. The bacterial answer to the treatments


Antibiotic targets
Cell wall
Beta-lactams
Vancomycin
DNA/RNA synthesis
Fluoroquinolones
Rifamycins
Folates synthesis
Trimethoprim
Sulphonamides
Cell membrane
Daptomycin
Protein synthesis
Linezolid
Tetracyclines
Macrolides
Aminoglycosides
Resistance mechanisms to antibiotics
Efflux
Fluoroquinolones
Aminoglycosides
Tetracyclines
Beta-lactams
Macrolides
Impermeability
Tetracyclines
Trimethoprim
Sulphonamides
Vancomycin
Target modification
Fluoroquinolones
Rifamycins
Vancomycin
Penicillins
Macrolides
Aminoglycosides
Inactivating enzymes
Beta-lactams
Aminoglycosides
Macrolides
Rifamycins

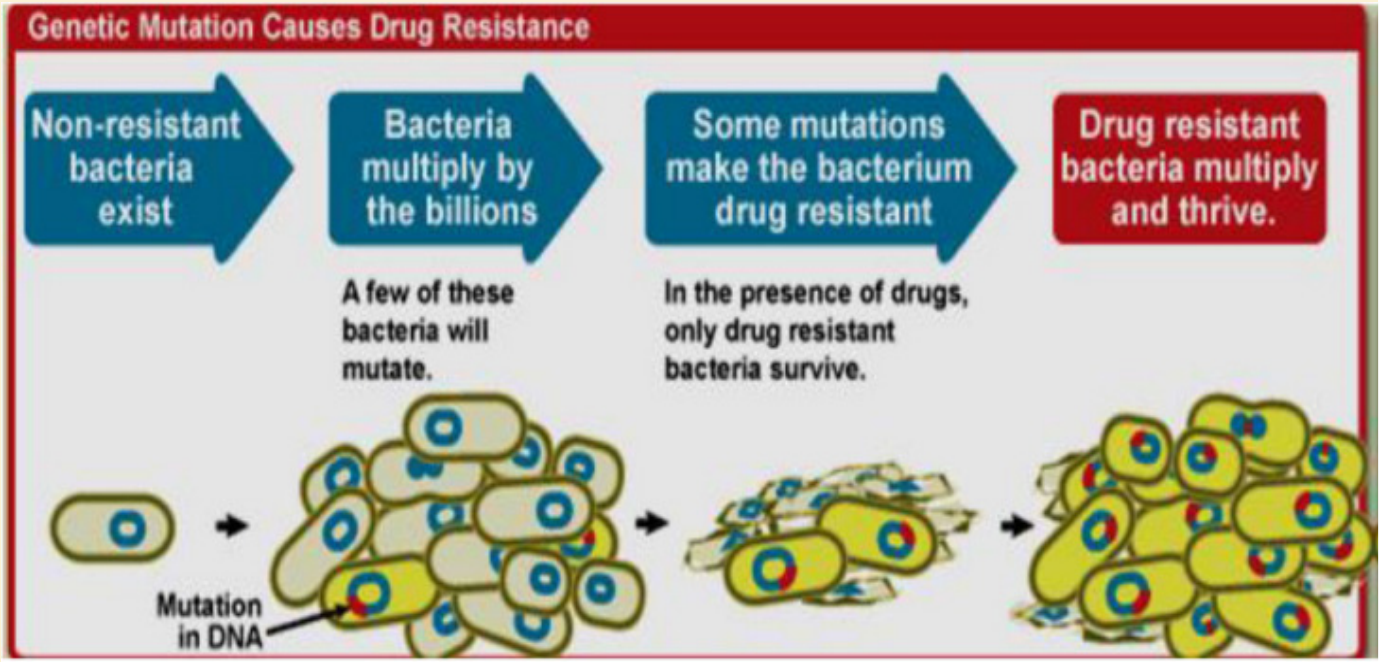
Antibiotic resistance

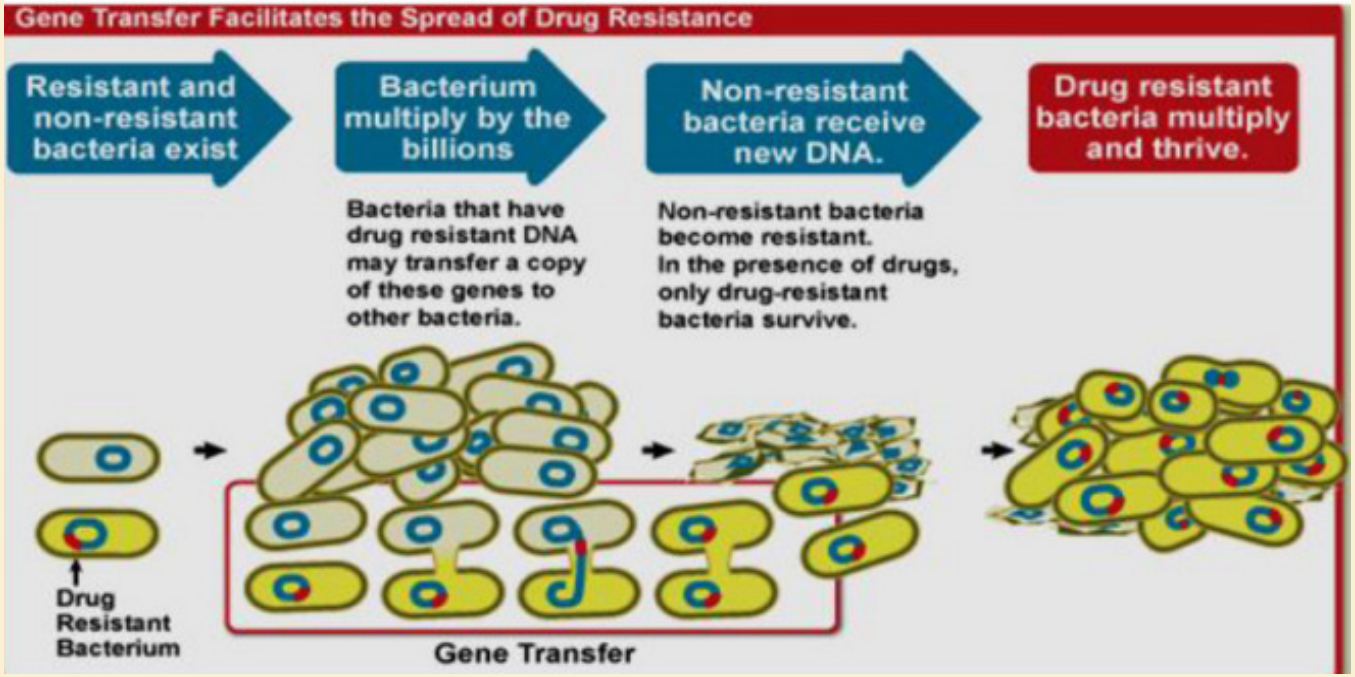
- Reported **everywhere in the world**.
- It **compromises the treatment** of infectious diseases.
- One of the **biggest threats to global health**.
- It can **affect any species**, regardless of age.
- It leads to **high medical costs and increased mortality**.

- Bacteria always can **adapt, evolve and acquire** resistance to antibiotics.
- The literature reveals **several ways** in which a bacterium may be resistant to an antibiotic.
- For example, those from the **National Institute of Allergy and Infectious Diseases (NIAID, 2012), present very suggestive stages and general factors** for the installation of drug resistance.

Installation of antibiotic resistance knowing three main stages:

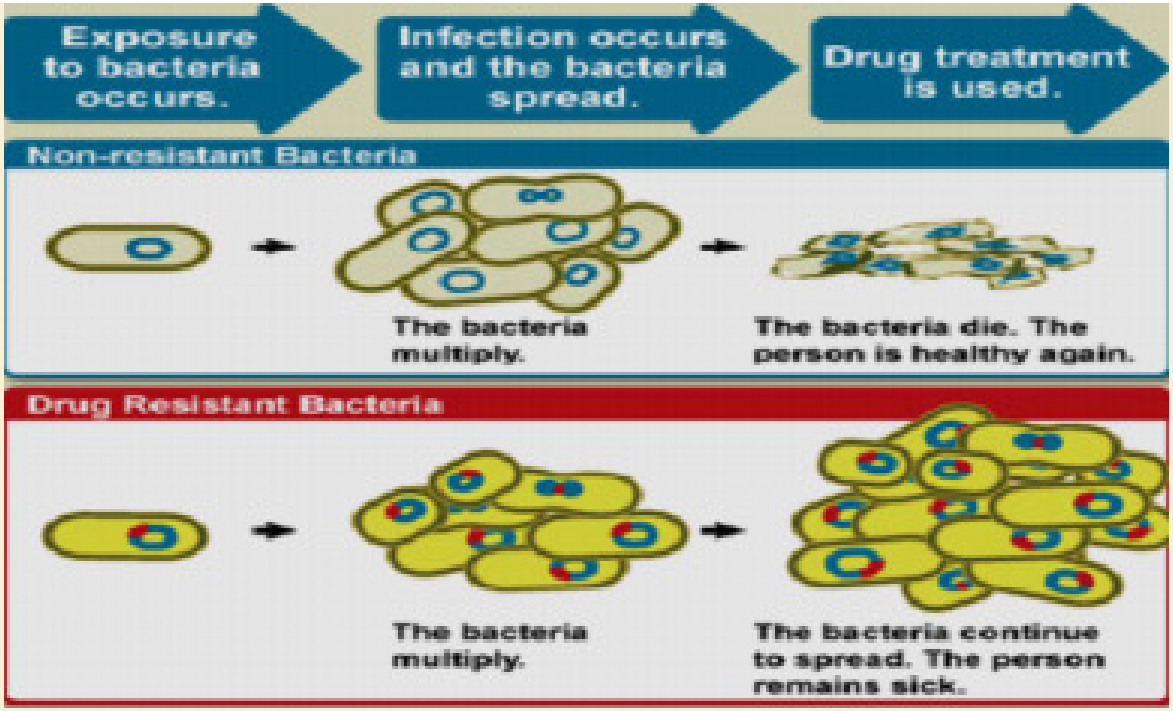
a) A genetic mutation can cause drug resistance.
Bacteria multiply logarithmically. Some of these bacteria will evolve and become mutants. Some of the mutations can determine the resistance of bacteria to drugs. In the presence of medicines, only resistant bacteria will be able to survive or even multiply.





- b) How gene transfer facilitates the spread of resistance.

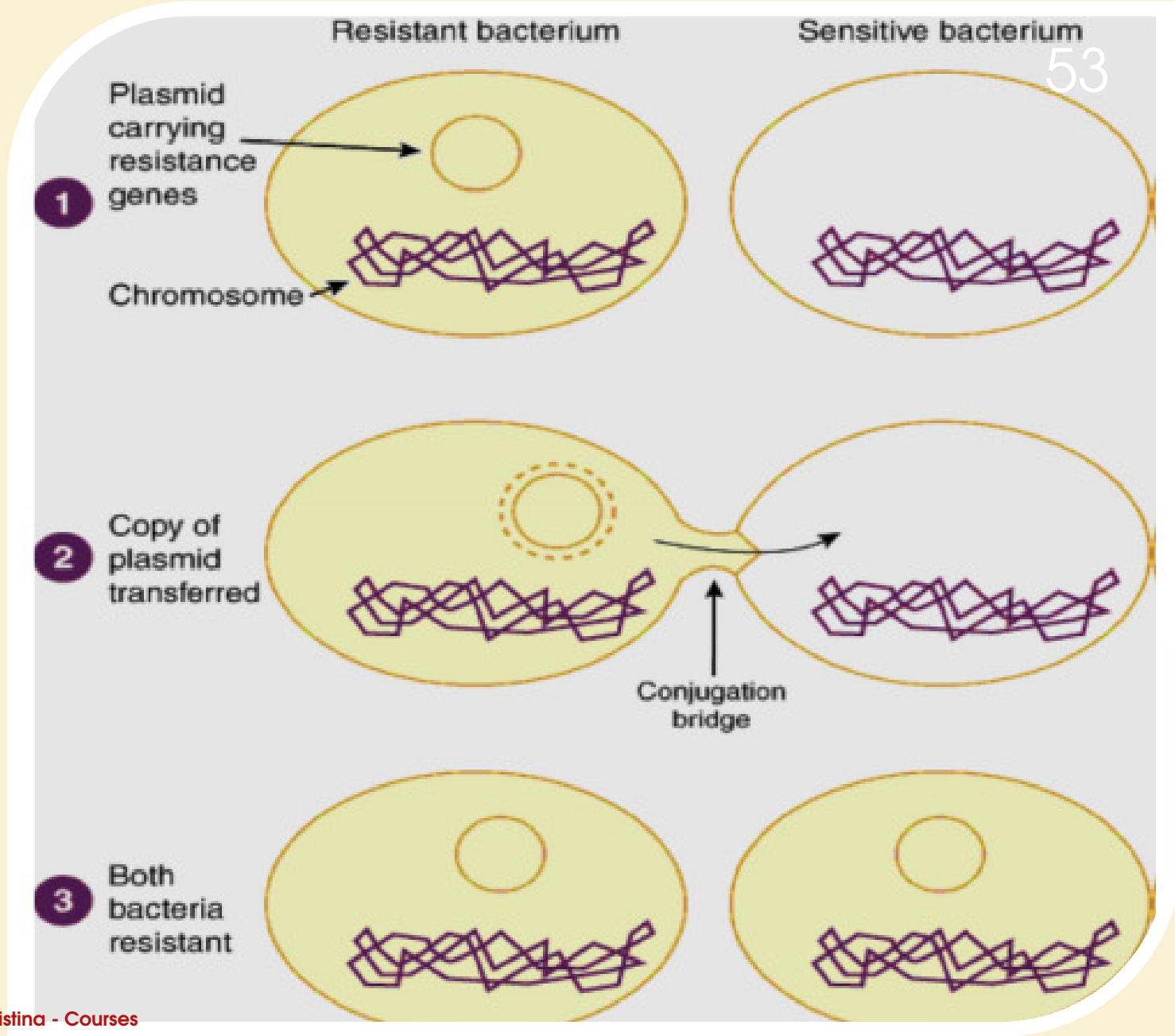
Bacteria multiply by the order of billions. Bacteria that possess the DNA of drug resistance can transfer a copy of these genes to other bacteria. Non-resistant bacteria will receive new DNA and thus become drug-resistant. In the presence of drugs, only resistant bacteria will survive and multiply and thrive.



- c) The difference between non-resistant and drug-resistant bacteria.

Non-resistant bacteria multiply, and with drug treatment, they die, while drug-resistant bacteria continue to multiply and spread even after the treatment is instituted. Energy and resources to maintain and reproduce. As a result, a larger genome will need more energy to keep it in operation and to duplicate it during reproduction

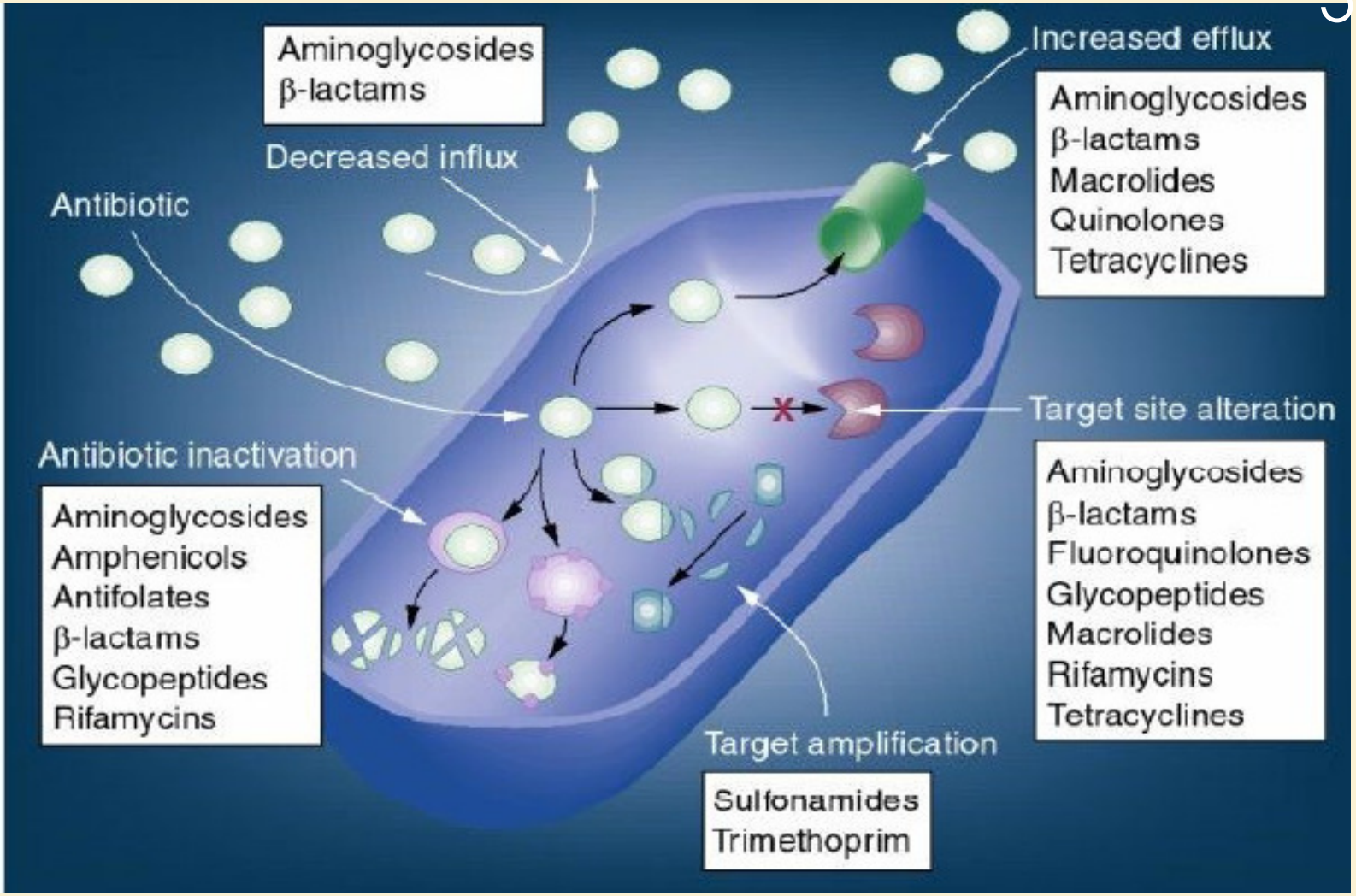
The general mechanism of resistance



- It is known that **bacteria grow much faster and in a much larger number than most other organisms**. ***In a single handful of mud are more bacteria than the entire population of the world.***
- The large bacterial population is followed by intense competition and operates on the **"best suited will survive"** principle.
- In bacteria, the **excess DNA** in this competition is considered **"ballast"** being rapidly eliminated, if a DNA sequence is not essential for survival or does not confer a selective advantage, it will be quickly moved and removed from the evolutionary genome of the bacterial population.
- In order for a gene and/or part of the bacterial genome to remain functional for a long time, it must help improve the survival and/or competitiveness of the bacteria.

- **the development and support of antibiotic resistance are usually dependent on the bacterial population,** being frequently exposed to non-lethal doses of antibiotics, of course without forgetting that some bacteria are naturally resistant to some antibiotics.
- In fact, this means that antibiotic resistance may occur in environments where bacteria are frequently exposed to antibiotics.
- **At the individual level,** this means that a person may develop an infection resistant to antibiotic treatment, following long-term or prophylactic treatment, as opposed to short-term treatments for acute infections.
- This may also mean **that bacteria may lose resistance to antibiotics that have not been used frequently.**

The main mode of action



- Bacteria become resistant to an antibiotic or group of antibiotics following several ***specific interactions***:

Inactivation of the drug or diversion of the metabolic pathway

It is the result of **enzymatic degradation** of the antibiotic by **bacterial enzymes such as beta-lactamases (penicillinases and cephalosporinases).** Inactivations may also occur in aminoglycosides, which may be acetylated or phosphorylated (by acetylases and phosphorylases).

Alteration of the enzyme target or structure

The receptor where the antibiotic usually acts **may change its affinity for the bacterium, and thus the receptor's response amplifies the bacterial activity** and implicitly cancels that of the drug.

Low accumulation of antibiotic in resistant bacterial cells

It occurs, for example, **in cancer cells,** when tetracycline accumulation decreases

Types of resistance

Natural antibiotic resistance (epigenetic adaptation)

- Bacteria that consistently challenge **sub-inhibitory levels of an antibiotic**.
- These are concentrations that are **too low to kill the bacterial population** but may develop temporary antibiotic resistance.
- This type of resistance is called **epigenetic adaptation**, and it does not produce genetic changes that can be permanently inherited by subsequent generations of bacteria.

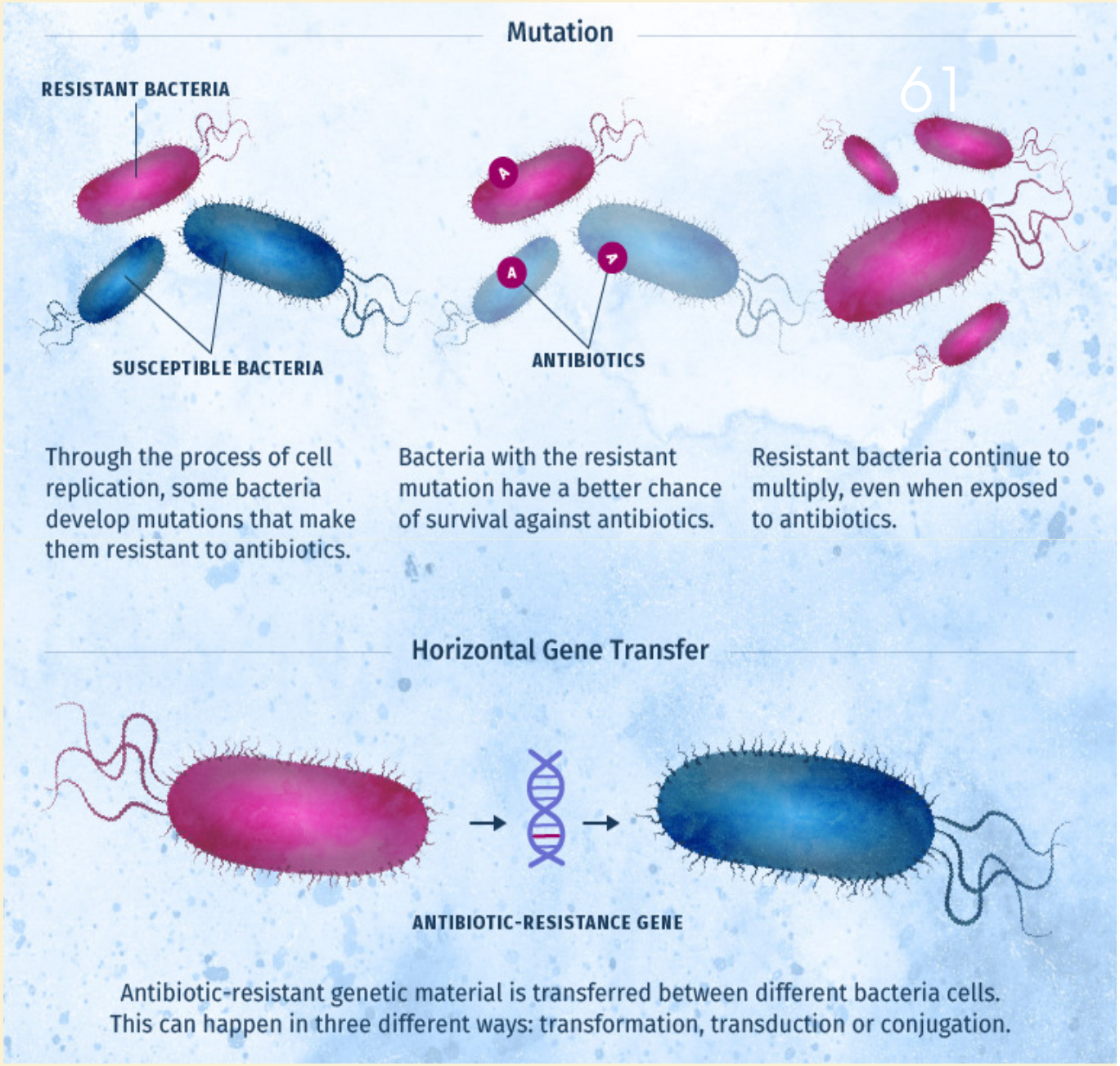
- This can be equated with an athlete who "develops his muscles" by physical training.
- Likewise, bacteria exposed to sub-inhibitory levels of an antibiotic can mobilize defenses, such as antibiotic expulsion pumps, enzymes to break them down, or they can simply be reduced by the permeability of the cell wall to reduce their exposure to antibiotic molecules.

As can be seen in the figure, antibiotics usually kill bacteria by **blocking or interfering with the activity of the enzymes** required for their metabolism (1). Bacteria have **"invented" the ply sly mechanism "the cunning adaptation"** to escape the antibacterial attack. Thus bacteria will **"spit" out their enzymes to lessen the effect of the antibiotic** (2). Then they will **shut down the cell wall to prevent the penetration of other antibiotics** (3) and **pump the antibiotic outside before it can kill** (4), the bacteria can further modify the targeted enzyme to deactivate the drug (5).

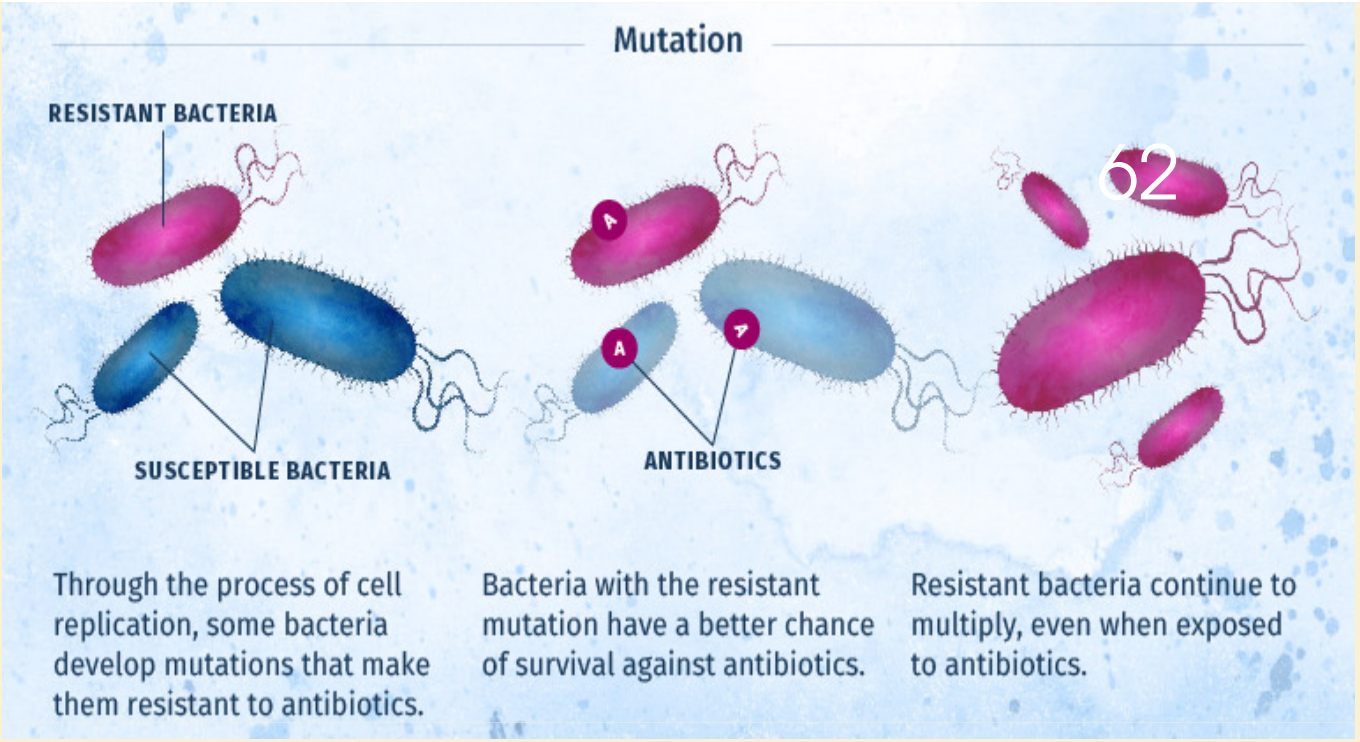
In this way the bacteria can easily switch to the most useful tools for their own survival and for other antibacterial invasions.

Acquired resistance

- Appears to **all antibiotics**, faster or slower.
- This is also why some antibiotics discovered, synthesized, extracted and researched therapeutically are not included in therapeutics.



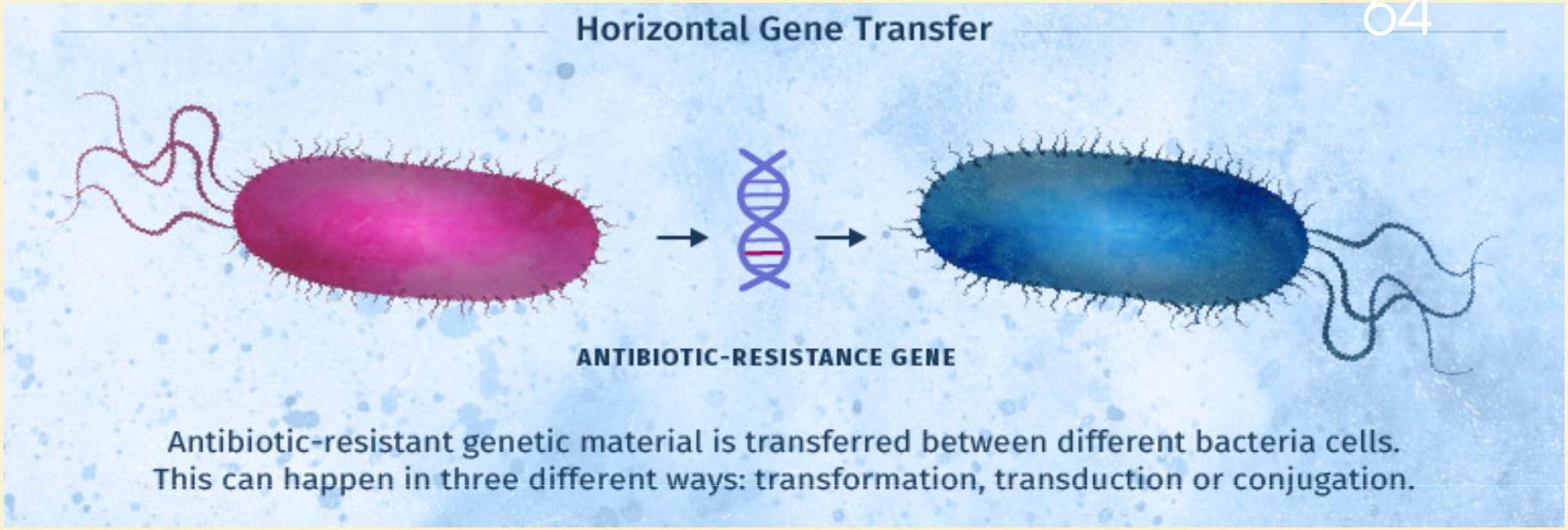
a. Genetic adaptation (through genetic mutations and selection)



- Numerous sources, most often of **exogenous origin, can cause resistance.**
- **UV radiation can cause permanent DNA damage and modification.**
- **Genetic mutations** are small changes to the genetic code that occur during DNA replication or as a result of exposure to ionizing radiation as mutagenic factors.

- Many genetic mutations occur in portions of the genome that are not essential for the body and do not change significantly in the functioning of the body. Some antibiotics are more likely to become less effective as a result of genetic mutations in target bacteria.
- Some antibiotics target the bacterial enzyme called **DNA gyrase.** The antibiotic binds to this enzyme, which prevents bacterial DNA replication. A unique mutation at a certain position in this enzyme can stop the effect of the antibiotic and allow the bacteria to become resistant to the antibiotic. Because of this, many antibiotics are not recommended in the long term, partly because of the increased likelihood that some bacteria will become resistant.

b. Genetic acquisition



- Bacteria can **acquire large parts of DNA** from other bacteria, viruses, and the environment. It is almost impossible for some bacteria to evolve by accident and encounter a gene or enzyme that offers resistance against a particular antibiotic (at least for weeks, months and years).
- Bacteria have many ways to acquire these large pieces of DNA that often contain many complete genes.

b. Genetic acquisition

▶ Plasmids

They are moving pieces of DNA (often circular) so bacteria can easily trade and acquire them from the environment, many bacteria have multiple plasmids.

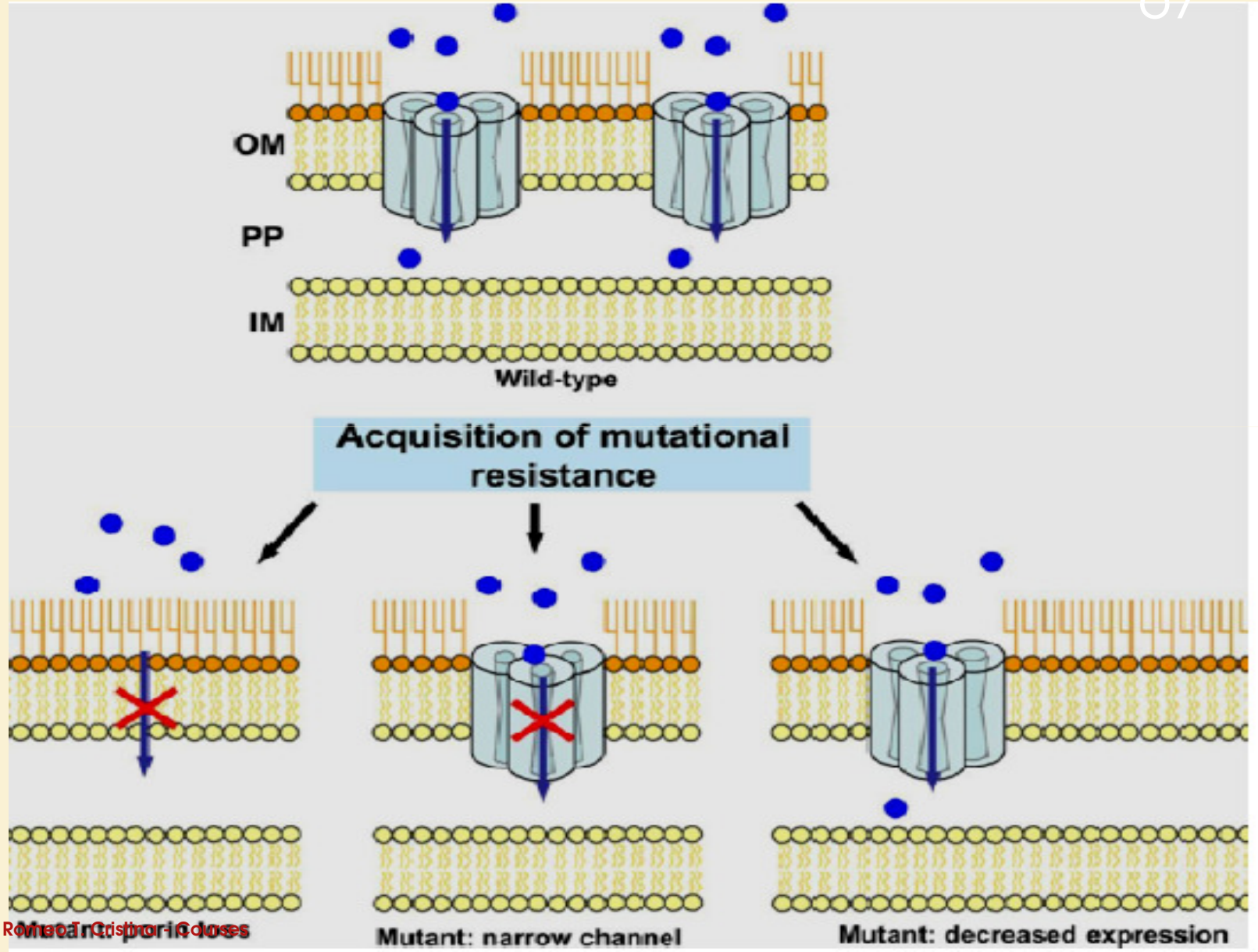
Plasmids may contain genes that inactivate an antibiotic.

The bacterium acquires a resistance plasmid, that is, a DNA fragment that carries extrachromosomal genes that can alter antibiotic resistance.

Genetic information present in plasmids is an important factor in the pathogenicity and invasiveness of bacteria, in the speed of occurrence of invasive pathogenic strains resistant to antimicrobial drugs and at the onset of symptoms.

- **Transposons.** There are sections of DNA that can jump from one place to another in the genetic code or even the genetic code of another organism
- **Bacteriophages.** Bacteria can be infected and these viruses can copy and paste into the genetic code, more specifically into the genome of infectious bacteria.
- **Conjugation** occurs if two bacteria are directly adjacent to each other, creating a direct connection by dividing the DNA
- **Naked DNA (free)** bacteria encompass the free DNA found in the environment. This DNA may be from dead bacteria, or part of a biofilm structure (some bacteria use DNA as a structure to anchor to a surface). Bacteria can use techniques to obtain DNA that help them to become resistant to a particular type and / or class of antibiotics.

The stages of mutation acquisition

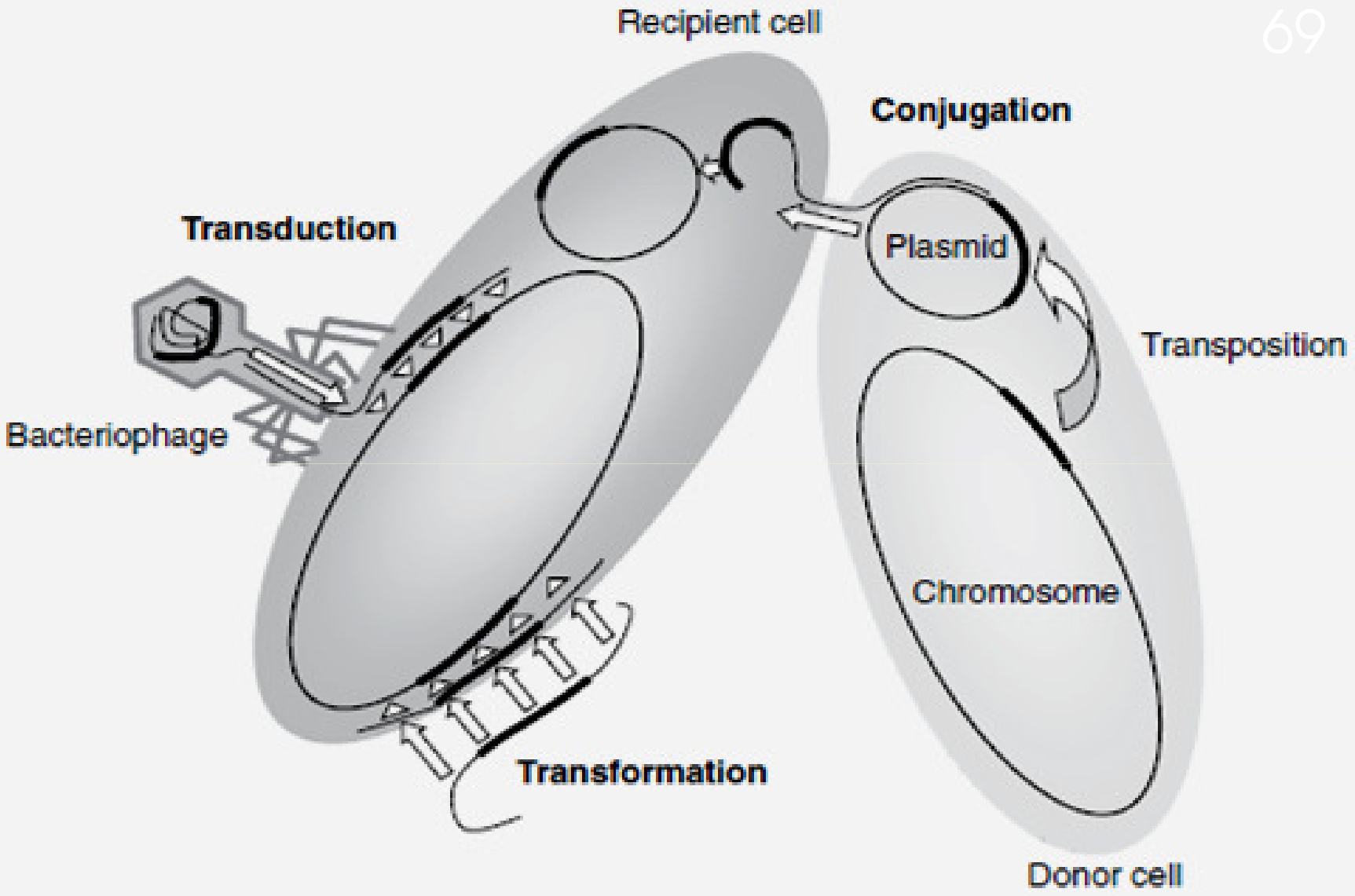


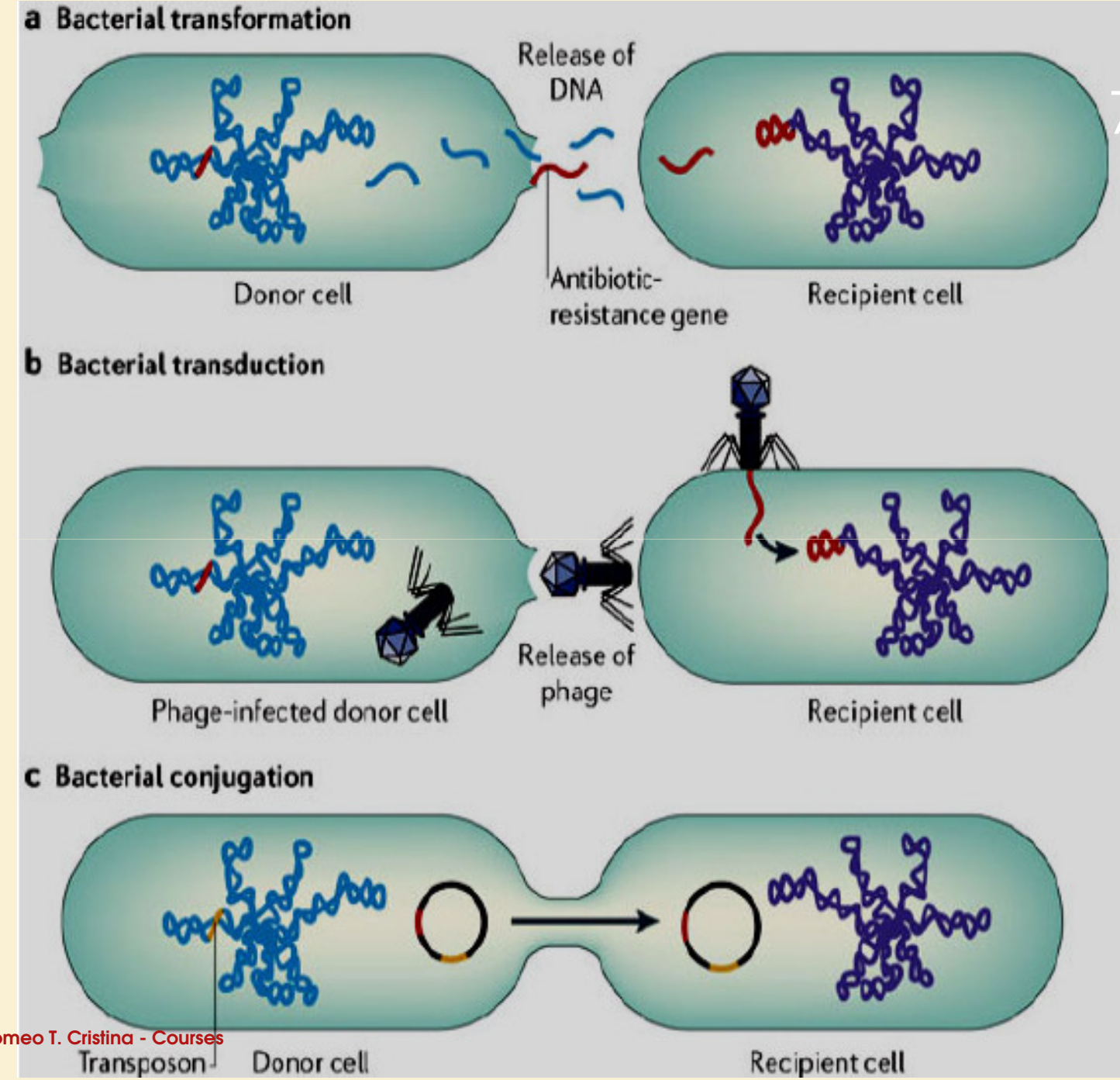
AMR-phases

- **Transformation** occurs when free DNA fragments, following the lysis of one organism, are taken over by another organism. The antibiotic resistance gene may be integrated into the chromosome or plasmid of the recipient cell.

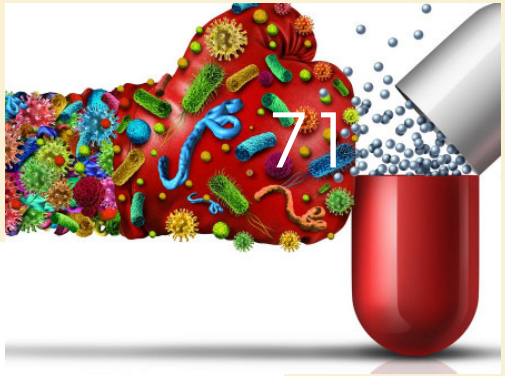
- **Transduction** is the phase in which the antibiotic resistance genes are transferred from one bacterium to another via bacteriophages and can be integrated into the chromosome of the recipient cell phenomenon known as lysogeny.

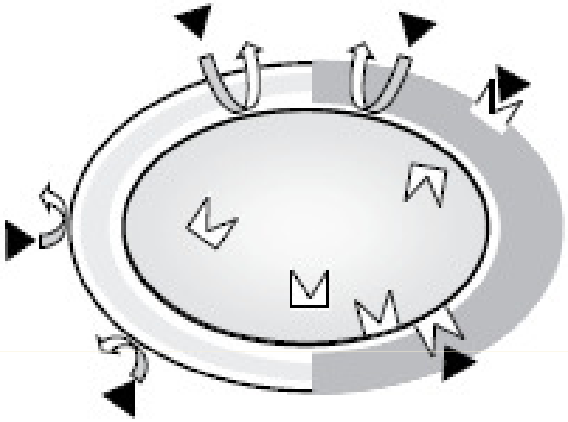
- **Conjugation** is the consequence of direct contact between two bacteria: the plasmids will form a mating bridge and the DNA is changed, which can lead to the acquisition of antibiotic resistant genes by the recipient cell.


Recipient cell
Conjugation
Transduction
Plasmid
Transposition
Bacteriophage
Chromosome
Transformation
Donor cell

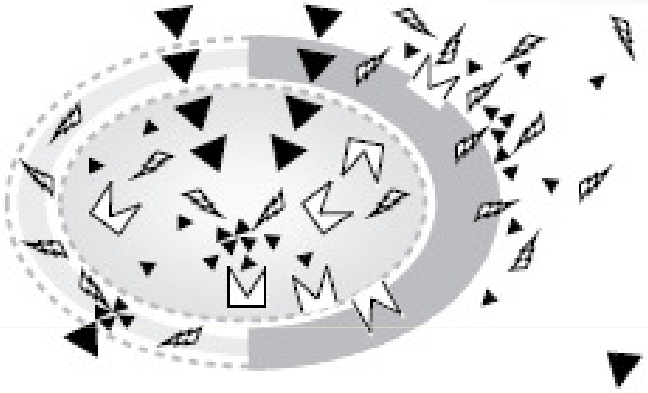

a Bacterial transformation
Release of DNA
Donor cell
Antibiotic-resistance gene
Recipient cell
b Bacterial transduction
Phage-infected donor cell
Release of phage
Recipient cell
c Bacterial conjugation
Transposon
Donor cell
Recipient cell

The mechanisms of resistance can be divided into four categories:

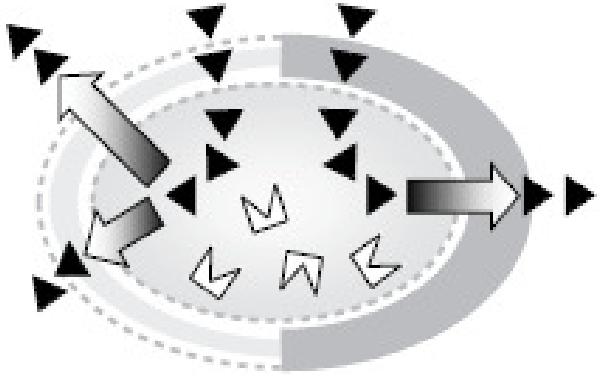




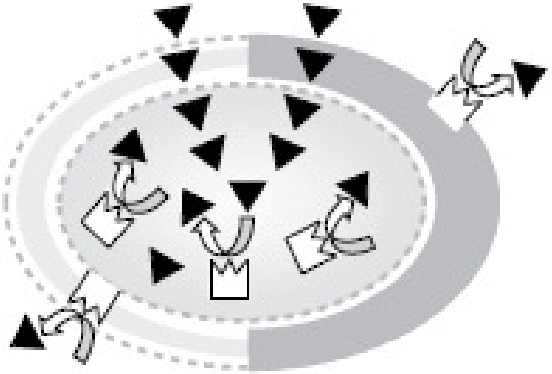
Reduced permeability



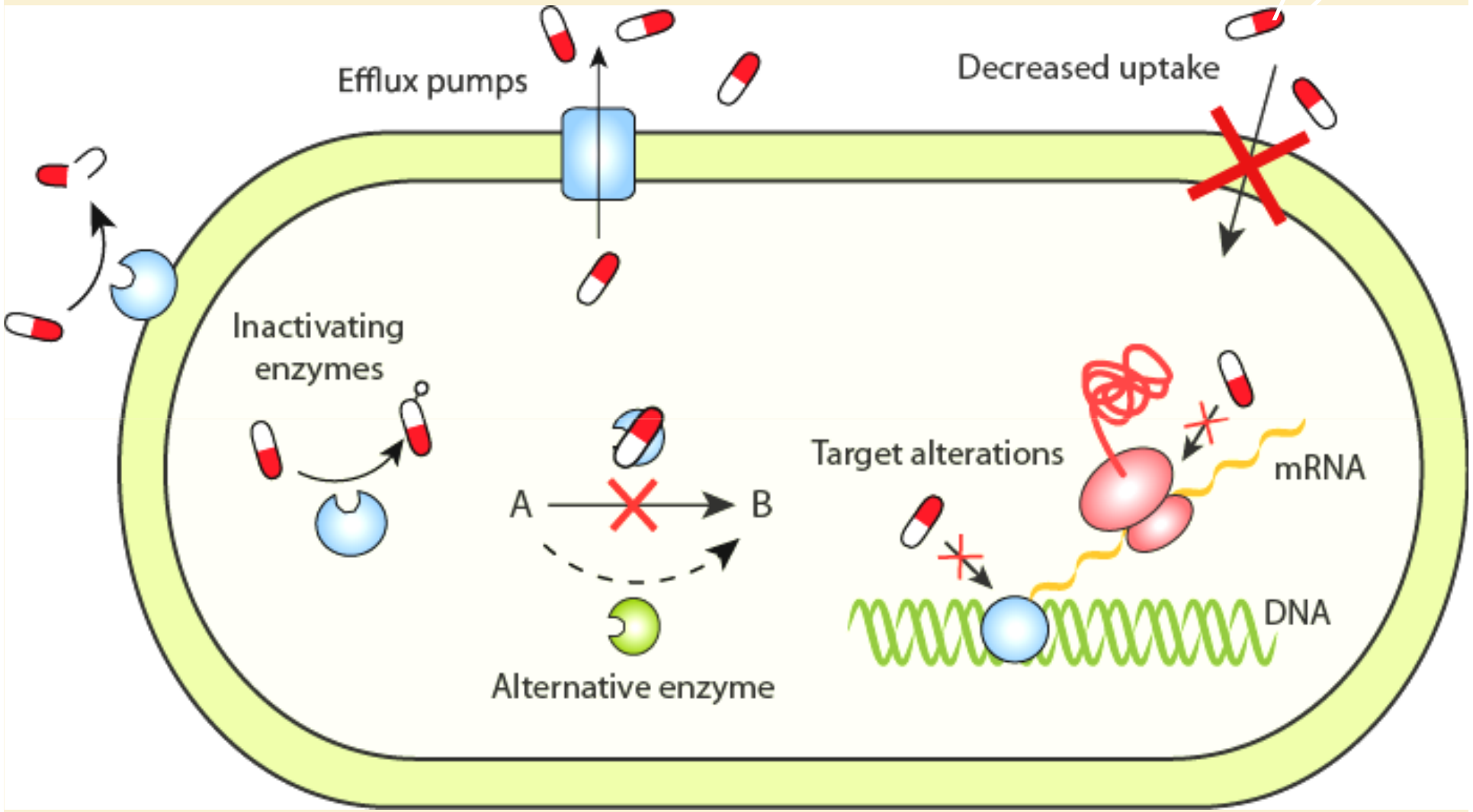
Antimicrobial agent modification



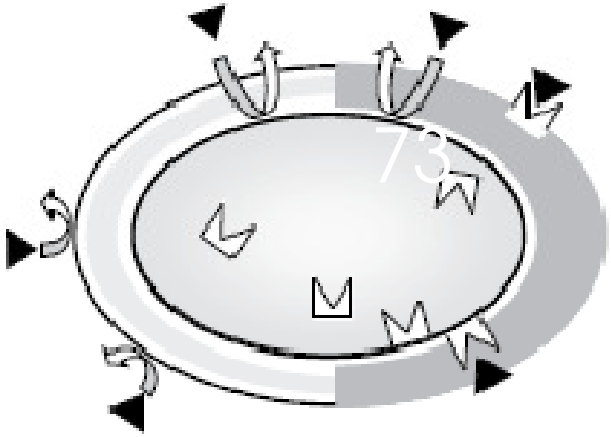
Active efflux



Target modification


Efflux pumps
Decreased uptake
Inactivating
enzymes
A
B
Alternative enzyme
Target alterations
mRNA
DNA

Decreased permeability



- it is the most common form of natural resistance.
- In this case, the antibiotic cannot penetrate the surface of the bacterium and therefore cannot reach the cell nucleus.
- In the case of Gram-positive bacteria, the bacterial wall is more permissive when entering antibiotics, but in the case of Gram-negative bacteria, it represents a difficult barrier to overcome and which varies according to the bacterial species.
- For example, the cell wall is more permeable for *Neisseria* and *Haemophylus, P. aeruginosa and Proteus indole-positive strains.* In the case of *Escherichia coli* and other enterobacteria, the specific protein (porine) will prevent the penetration of hydrophilic antibiotics with a molecular weight of up to 650 Daltons.

- Examples of **Gram-negative bacillus resistance** due to decreased permeability in the case of **penicillin G, erythromycin, clindamycin and vancomycin resistance**
- Resistance of streptococci, *Pseudomonas aeruginosa* and other anaerobic bacteria to **aminoglycosides**

Modification / inactivation of antibiotics

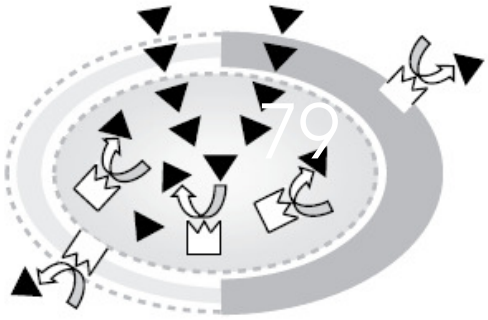
Antimicrobial agent modification

- is the most common mechanism of acquired resistance and is largely determined by the production of beta-lactamase enzymes
- Beta lactamases are a group of enzymes produced by Gram-positive, Gram-negative aerobic and anaerobic bacteria capable of hydrolyzing the beta-lactam ring and thus inactivating the appropriate antibiotic.

- This specific mechanism has long been shown to be an important factor in germ resistance such as *Staphylococcus aureus, H. influenzae, N. gonorrhoeae, Bacteroides fragilis*, and some enterobacteria.
- Genetic information for the synthesis of these enzymes may be contained in a chromosome or plasmid and its production may be a characteristic of constant bacterial production, although it may also be induced in the presence of a suitable substrate.

- **Beta-lactamase,** based on substrate profile and response to enzyme inhibitors, are classified into five large groups. In practice, the most important are groups I and III.
- **Group I lactamase** is produced in significant quantities, in the presence of antibiotics, encoded by chromosome genes and distributed among *Enterobacteriaceae* strains. These lactamases are responsible for the resistance of Gram-negative nosocomial strains to cephalosporins.
- **The lactamase III enzyme category is active on penicillins and cephalosporins** and is almost always plasmid-encoded, this group includes beta-lactamase TEM present in *enterobacteria, H. influenzae, and N. gonorrhoeae*.

- Among the anaerobic beta-lactamase-producing bacteria is *Bacteroides fragilis*, which produces a cephalosporinase, inactivated by clavulanic acid. Sulbactam and clavulanic acid are capable of inhibiting beta-lactamases, in essence, those mediated by plasmids when combined with certain antibiotics, amoxicillin, ampicillin, ticarcillin, and others. Recently, beta-lactamase bacterial strains have been identified that can hydrolyze new beta-lactam.
- Included in this group are **enzymes** isolated from **K. pneumoniae-mediated plasmid** strains that can hydrolyze cefotaxime and other third-generation cephalosporins, as well as aztreonam and chromosome-mediated enzymes present in *Pseudomonas maltophia* and *Enterobacter cloaca* strains, *Serratia marcense*, and *Bacteroides fragilis* capable of hydrolyzing imipenem and meropenem.

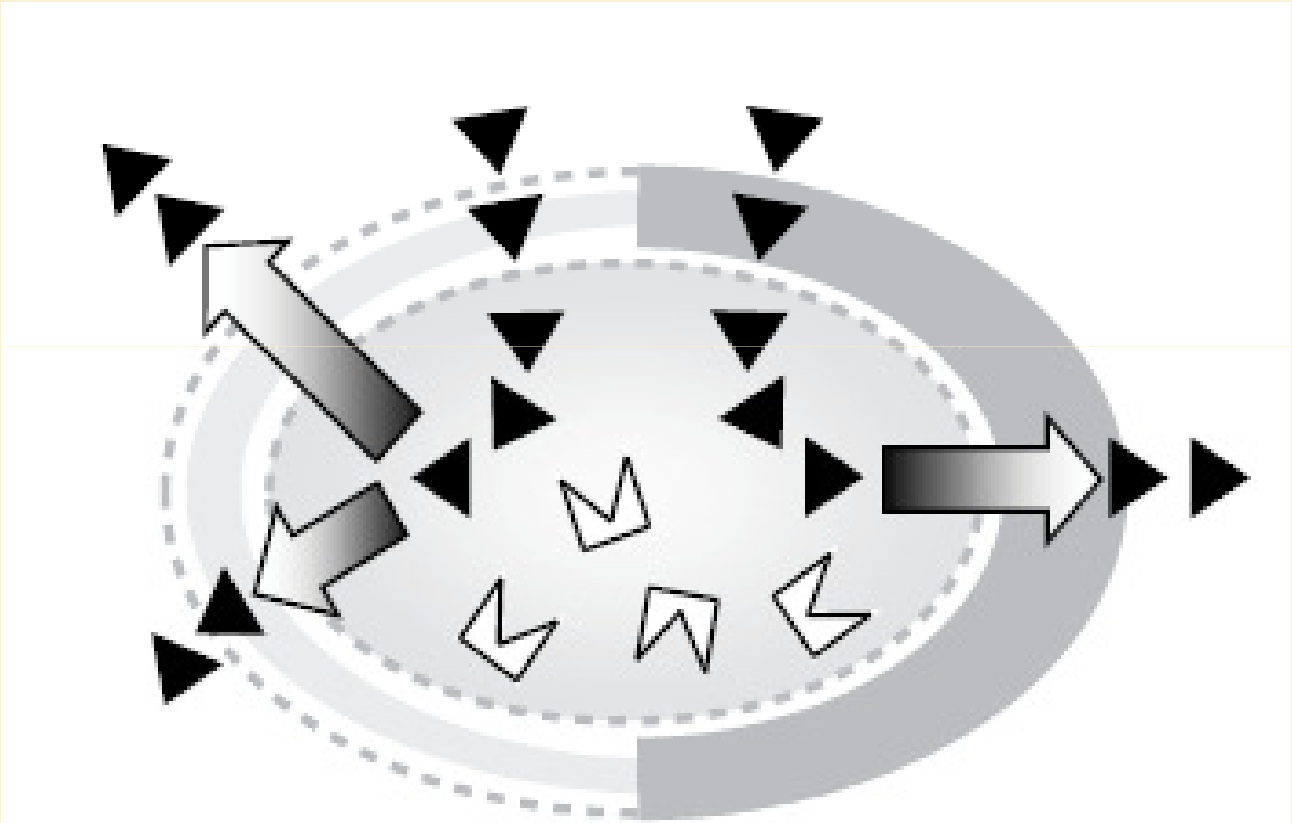


Changes to the antibiotic site of action

- These resistance mechanisms refer to changes in the structure or stages of metabolism for which drugs exert their action. This will be achieved either by increasing the concentration of a competitive substance or by modifying different alternative bacterial structures.
- **Tolerance**

It is not considered a classic mechanism of resistance in practice, and can behave as such. It is attributed to the selection of deficient mutants in autolytic systems. Likely, high doses designed to reach levels well beyond the CMI of microorganisms would reduce the selection of these subpopulations when the duration of treatment is necessary.

Efflux pump



Active efflux

Enzymatic alteration

Class of antimicrobial agent	Activity	Phenotypic determinant of resistance	Bacterial genera representative
β-Lactam	-Cidal	Penicillinases	*Staph. aureus*
		Carbapenemases (KPC-1, KPC-2, KPC-3)	*E.coli, K.pneumonie*
		Cephalosporinases Class C beta-lactamase. This enzyme breaks the beta-lactam antibiotic ring open and deactivates the molecule's antibacterial properites.	*E. coli, Salmonella, Klebsiella*
		Class A beta-lactamase. This enzyme breaks the beta-lactam antibiotic ring open and deactivates the molecule's antibacterial properites.	*E. coli*
		Class D beta-lactamase. This enzyme breaks the beta-lactam antibiotic ring open and deactivates the molecule's antibacterial properites.	*E. Coli*

Enzymatic alteration

By class of antimicrobial agent	Activity	Phenotypic determinant of resistance	Bacterial genera representative
Aminoglycoside Resistance–Modifying Enzymes	-Cidal	aminoglycoside-modifying enzymes – aminoglycoside N-acetyltransferases (Plasmid, transposon, integron) O-phosphotransferases O-nucleotidyltransferases	*S. aureus* *S. marcescens, E. coli,* *A. baumannii, K. pneumoniae,* *K, oxytoca,* *E.coli (Kaster et al., 1983)* *Streptomyces hygroscopicus (Zalacain et al., 1986)* *S. griseus (Distler et al., 1987)* *S. enterica, P. aeruginosa, E. Coli (Steiniger-White et al., 2004)* *K. pneumoniae, Salmonella spp., E. coli,* *K. pneumoniae, Salmonella spp., E. coli, Staphylococcus epidermidis, (Gill et al., 2005)* *E. faecium, Streptococcus suis, S. Aureus (Holden et al., 2009)*

Enzymatic alteration

By class of antimicrobial agent	Activity	Phenotypic determinant of resistance	Bacterial genera representative
Lincosamides (Lincomycin) Inactivating enzymes	-static	Streptogramin A O-acetyltransferase Streptogramin B hydrolase	*Staphylococcus aureus*

Enzymatic alteration

By class of antimicrobial agent	Activity	Phenotypic determinant of resistance	Bacterial genera representative
Macrolides	-Static	Erythromycin esterase type I Erythromycin esterase typeII Aminoglycoside N-acetyltransferase, which modifies aminoglycosides by acetylation	*E.coli*
		rRNA adenine N-6-methyltransferase, which can methylate adenine at position 2058 of 23S rRNA, conferring resistance to erythromycin	*S. aureus*

Enzymatic alteration

By class of antimicrobial agent	Activity	Phenotypic determinant of resistance	Bacterial genera representative
Chloramphenicol	Mainly bacteriostatic, It becomes - cidal against pathogens	chloramphenicol acetyltransferases (CATs) Group A chloramphenicol acetyltransferase, which can inactivate chloramphenicol. Group B chloramphenicol acetyltransferase, which can inactivate chloramphenicol. Also referred to as xenobiotic acetyltransferase Group A chloramphenicol acetyltransferase	*Salmonella typhimurium* *Pseudomonas aeruginosa* *E.coli* *E.coli* *S. Aureus*

Decreased permeability

Aminoglycoside (Kanamycin) Phenicols (Florfencicol)	Cidal		
Cephalosporins (first-generation) - Reduced permeability			
Folate Pathway Inhibitors (Sulfonamides)	Static		
Macrolides (15-membered ring)	Static		
Quinolones/Fluoroquinolones	Cidal	DHFR	*Staphylococcus aureus*

Efflux of drug

Aminocyclitols, Aminoglycoside (Streptomycin)	Cidal	Resistance-nodulation-cell division transporter system. Multidrug resistance efflux pump.	87 *E. coli*
Cephalosporins (first-generation)			
Macrolides (15-membered ring) Alteration of 23S rRNA, r-protein L4; modification of drug/ribosome structure; efflux.	Static	Plasmid, Transposon Resistance-nodulation-cell division transporter system. Multidrug resistance efflux pump Resistance-nodulation-cell division transporter system. Multidrug resistance efflux pump. Macrolide-specific efflux system. Major facilitator superfamily transporter, Macrolide-Lincosamyde-Streptogramin B efflux pump.	*Staphylociccus epidermdtis* *E. fecalis* *Str. pyogenes* *St. aureus* *E.coli, E.coli* *S. Aureus*
Cloramfenicol	Mainly bacteriostatic, It becomes -cidal against pathogens	Major facilitator superfamily transporter, chloramphenicol efflux pump	*E.coli*
Quinolones	Cidal	Major facilitator superfamily transporter. Multidrug resistance efflux pump	*E.coli*
Tetracyclines	Static/Cidal	Major facilitator superfamily transporter, tetracycline efflux pump	*Proteus* *Enterobacter* *Pseudomonas* *Salmonella* *Escherichia*
Trimethoprim	Static alone Cidal with sulfonamides	Major facilitator superfamily transporter, chloramphenicol efflux pump.	*S. aureus*

Alteration of target site

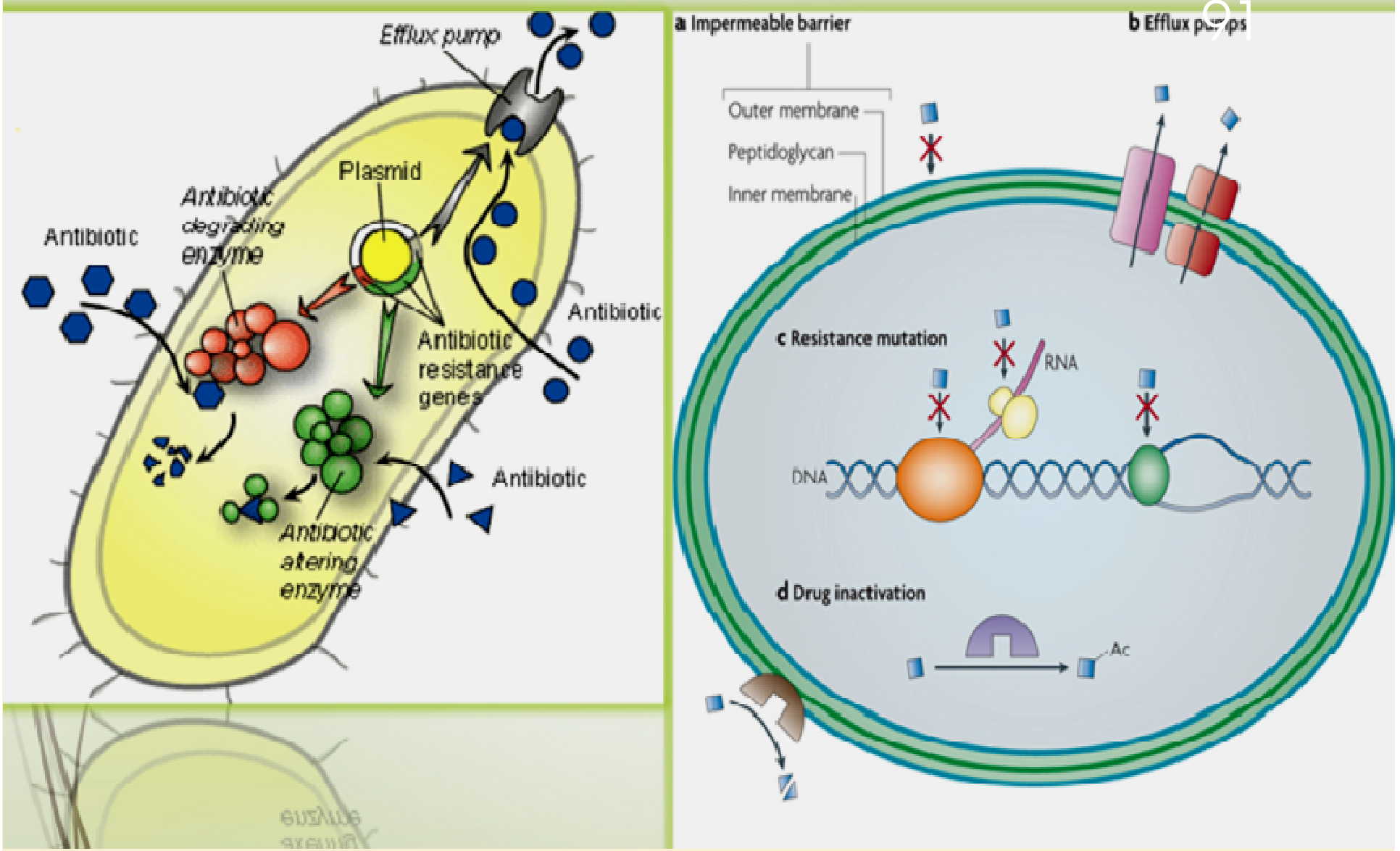
Aminoglycoside Alteration of 16S rRNA, r-protein S12; modification, of drug structure.	Cidal		
Cephalosporins (first-generation) - Altered pencillin-binding proteins			
Quinolones/Fluoroquinolones - Target site (DNA gyrase, topoisomerase IV) mutation	Cidal		
Tetracyclines - Target site (ribosome) mutation	Static/cidal	Ribosomal protection protein, which protects ribosome from the translation inhibition of tetracycline	*E. coli*

Protection of target site

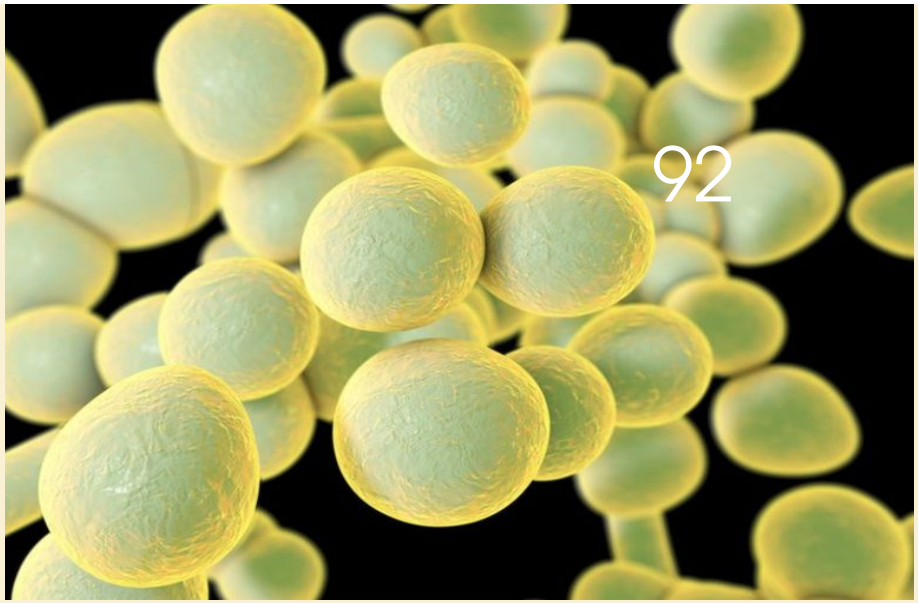
Glycopeptides (vancomycin) - Target site (cell wall) resistance	Cidal		*Staphylococcus aureus (vanA) Weigel et al., 2003*
Quinolones	Cidal	Pentapeptide repeat family, which protects DNA gyrase from the inhibition of quinolones	*E. coli*

Overproduction of target

Phenicols (Chloramphenicol) **Target site (ribosome) modification/ mutation**	Mainly bacteriostatic, It becomes -cidal against pathogens


Efflux pump
Plasmid
Antibiotic degrading enzyme
Antibiotic
Antibiotic resistance genes
Antibiotic
Antibiotic
Antibiotic altering enzyme
a Impermeable barrier
Outer membrane
Peptidoglycan
Inner membrane
b Efflux pumps
c Resistance mutation
RNA
DNA
d Drug inactivation
Ac

Antifungal resistance

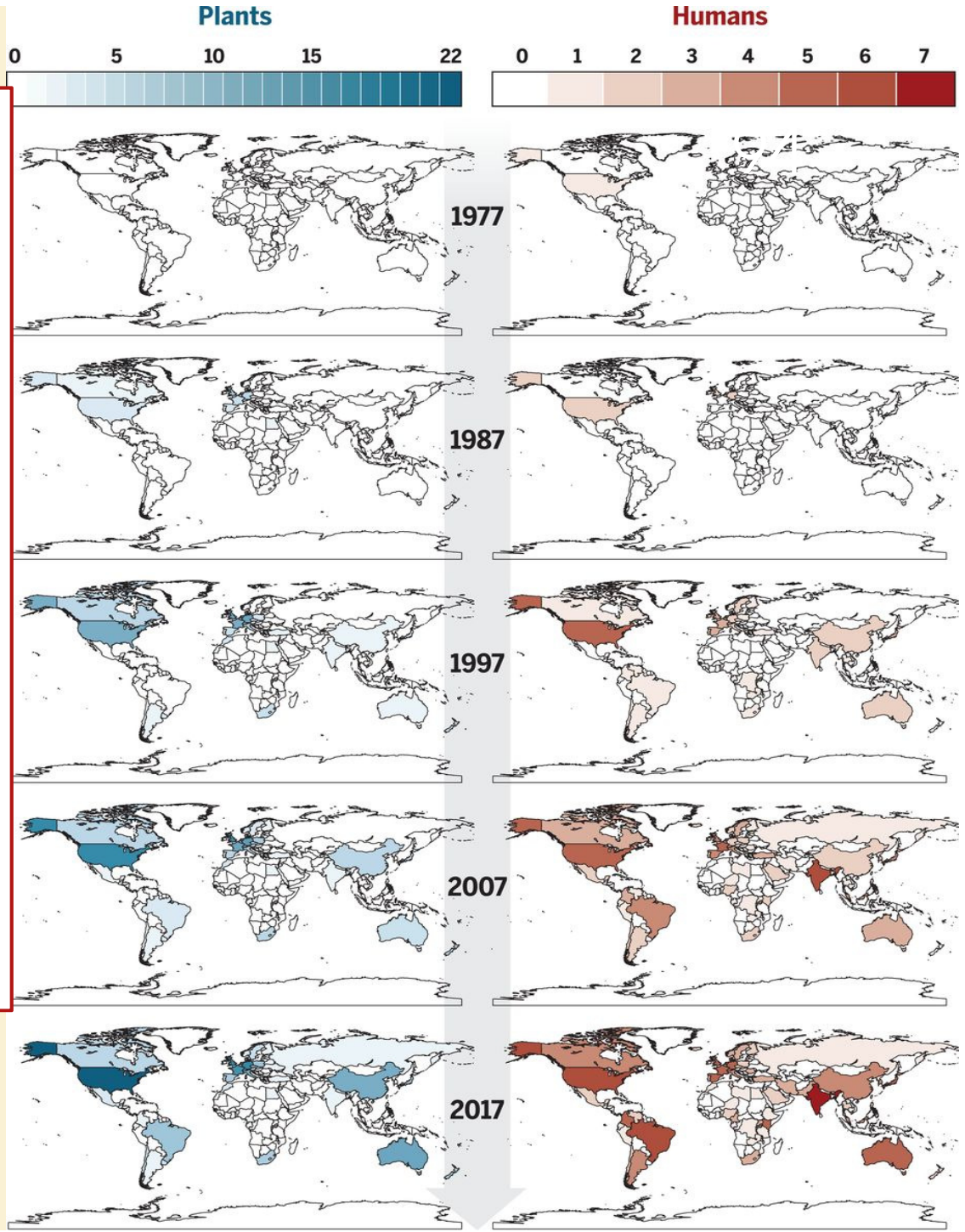


- Although the prevalence of antifungal resistance is not at the levels observed for some bacteria against different antibiotics, treatment options for invasive fungal infections are limited.
- New treatment strategies are needed to resolve this problem, in addition to overcoming the toxic effects / adverse effects and drug interactions associated with currently available antifungals, which may limit the effectiveness of the therapy.

- Resistance to antifungal drugs can be **intrinsic or acquired**.
- Intrinsic resistance is an inherited characteristic of the species or strain.
- In contrast, acquired resistance occurs when a previously sensitive isolate develops a resistant phenotype, usually as a result of prolonged antifungal treatment.
- The precise mechanism associated with the acquired resistance depends on the mode of action of the class of antifungal drugs
- Unlike bacterial cells, intact fungal cells do not readily pick up exogenous DNA.

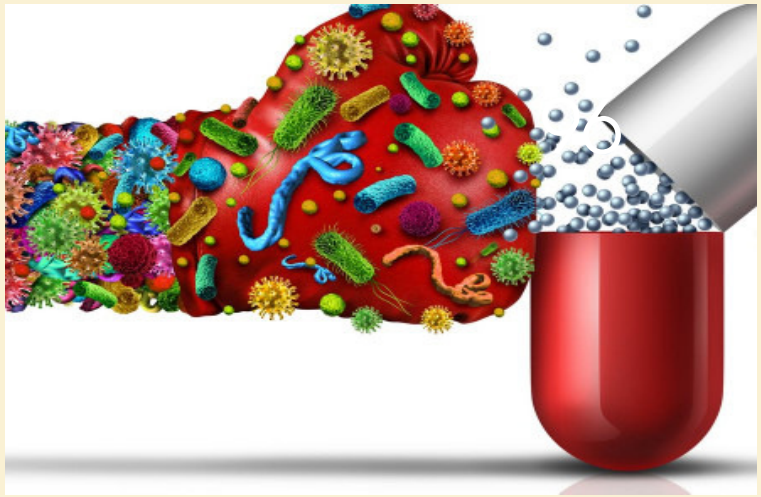
Low incidence of fungal infections in past

Increased incidence of fungal infections has led to the massive use of antifungals, massive research on antifungal agents, and increased resistance to antifungals as evidenced by the increase in publications since the 1960s.

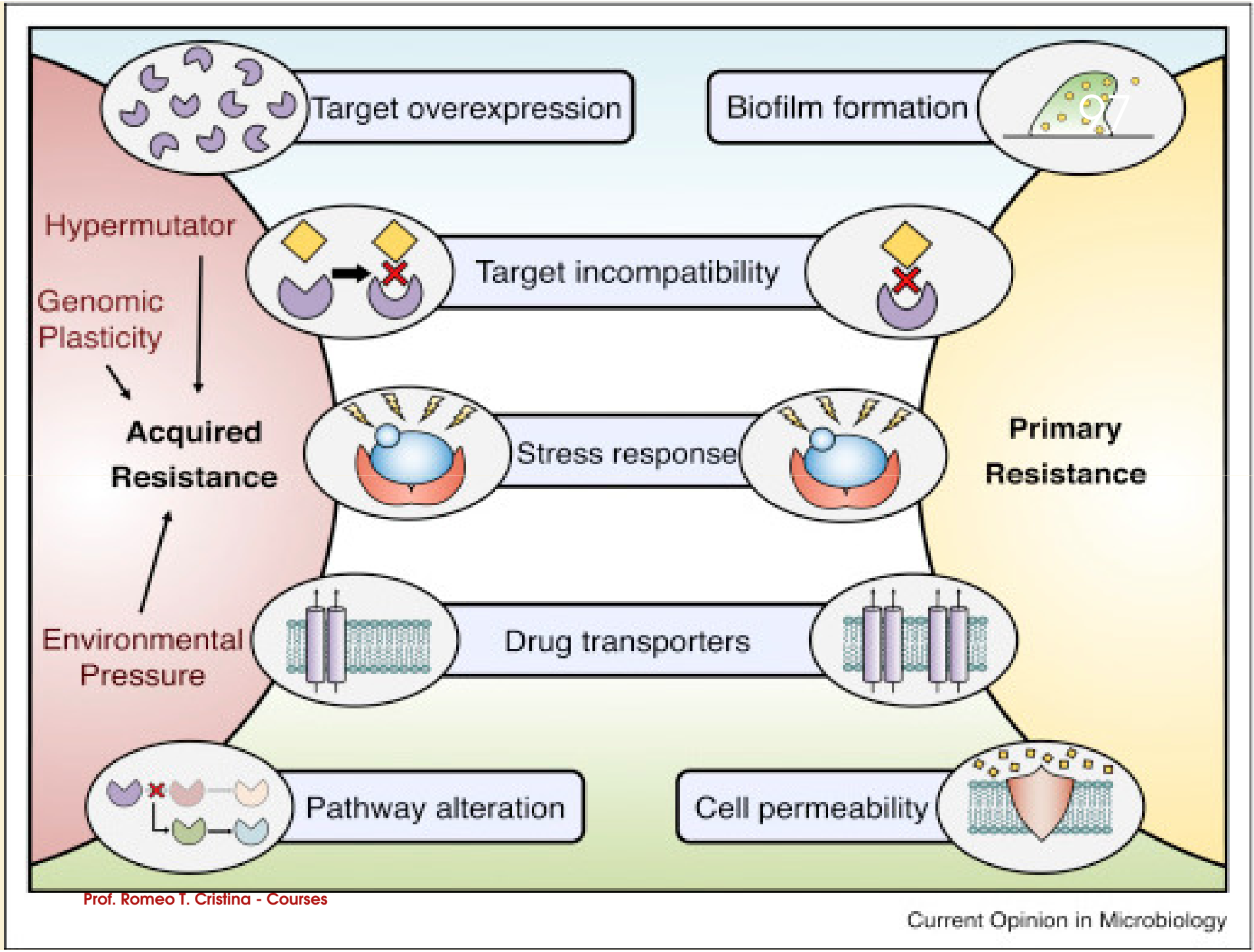


- **Our limited antifungal arsenal** is further threatened by the development of many drug-resistant fungus strains and the emergence of intrinsically resistant pathogens.
- **Microorganisms** develop mechanisms to counter the **fungicidal or fungistatic effects** of all antifungal classes.

Mechanisms of antifungal resistance



- Fungi are based on **four major mechanisms**, namely:
- reducing the accumulation of the drug in the fungal cell
- elimination of antifungals by efflux pumps
- decreasing affinity of the target drug
- changes in metabolism to counterbalance the effect of the drug

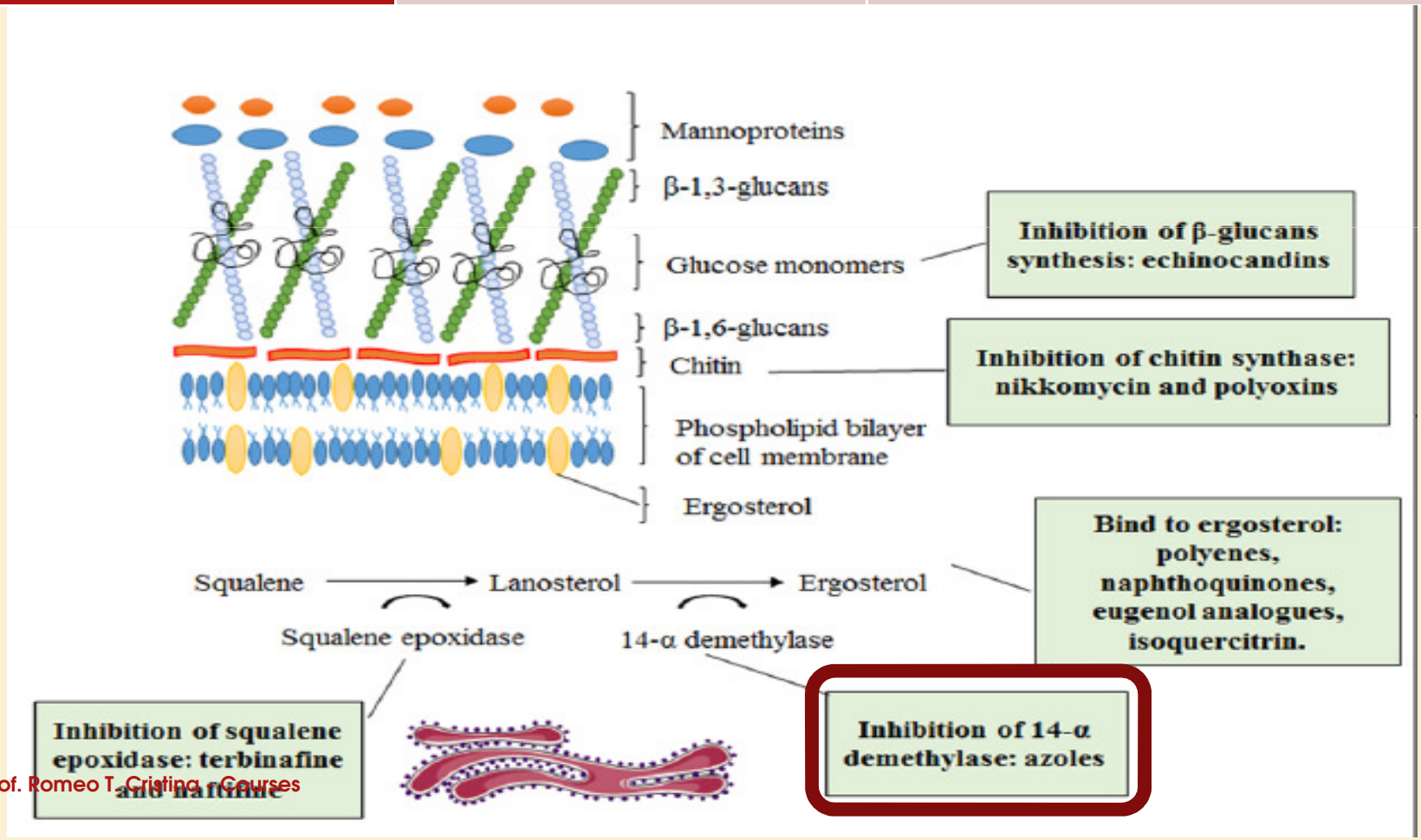

Target overexpression
Biofilm formation
Hypermutator
Genomic Plasticity
Target incompatibility
Acquired Resistance
Stress response
Primary Resistance
Environmental Pressure
Drug transporters
Pathway alteration
Cell permeability
Current Opinion in Microbiology

Antifungal drugs	Site of action	Mechanism of resistance
Polyenes (Amphotericin B, Nistatin)	inhibition of ergosterol biosynthesis	- absence of ergosterol (mutation to ERG3 or ERG6); -decreased ergosterol content in cells;
Azoles **Imidazoles: Clotrimazole, Enilconazole, Ketoconazole, Miconazole** **Triazoles: Fluconazole. Itraconazole, Posaconazole)**	-Inhibition of the function of cytochrome P450: 14α-lanosterol demethylase (ERG11) -interfering of ergosterol synthesis	- Multidrug-mediated efflux; - decreased affinity for Erg11p through mutations; - growth of ERG11; - alterations of ergosterol biosynthesis;
Allylamine (Terbinafine)	Inhibition of squalene oxidase	unknown
5-floropirimidine (Flucytosine)	DNA and RNA synthesis	- Deficiency of cytosine permeability -deficiency or lack of enzymes involved in the metabolism of flucytosine -regulation of the pyrimidine biosynthetic pathway
Echinocandins (Caspofungins)	Inhibits β-1,3 glucan synthetase	- modification of the affinity of echinocandins for β (1,3) - glucan synthetase

- **The molecular mechanisms of primary resistance** are extremely diverse and specific to each antifungal agent.
- At present, **numerous data on the resistance of pathogenic fungi** to the action of various medicinal preparations are accumulated.

One of the most widespread mechanisms of resistance to the action of antifungal drugs is overexpression of enzymes involved in ergosterol biosynthesis: the cytochrome P450-dependent lanosterol 14a-demethylase enzyme (known as Erg11 in yeasts, eg *Candida albicans*, *Cryptococcus neoformans* and as Cyp51A in mycelial fungi, e.g. (*Aspergillus fumigatus*)

Antifungal drugs	Site of action	Mechanism of rezistance
Azoles **Imidazoles: Clotrimazole, Enilconazole, Ketoconazole, Miconazole** **Triazoles: Fluconazole. Itraconazole, Posaconazole**	-Inhibition of the function of cytochrome P450: 14α-lanosterol demethylase (ERG11) -interfering of ergosterol synthesis	- Multidrug-mediated efflux; - decreased affinity for Erg11p through mutations; - growth of ERG11; - alterations of ergosterol biosynthesis;



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Medical Mycology Case Reports

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Azole resistance of *Malassezia pachydermatis* causing treatment failure in a dog

Martina Angileri,[a] Mario Pasquetti,[b] Michela De Lucia,[a] and Andrea Peano[b,*]

An azole-resistant isolate of Malassezia pachydermatis

Article *in* Veterinary Microbiology 149(1-2):288-90 · October 2010 *with* 43 Reads
DOI: 10.1016/j.vetmic.2010.10.010 · Source: PubMed

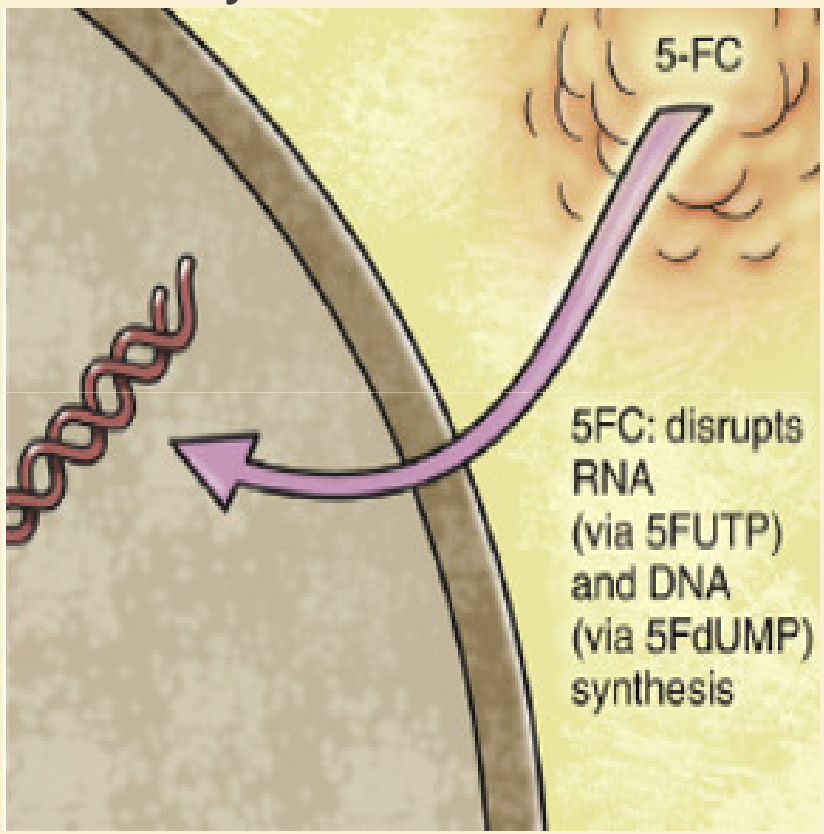
- The azoles bind to the ferric fragments of the heme-binding areas and block the natural substrate of the enzyme - lanosterol, thus disrupting the biosynthesis process.
- Mechanisms of resistance to azoles targeting the cell membrane may occur by overexpression or modification of the target of the preparation, upregulation of transporter activity, or induction of cellular changes that reduce drug toxicity or induce tolerance to the drug.

Susceptibility study: Yeasts of the Malassezia genus are highly susceptible to itraconazole and posaconazole and less to fluconazole, regardless of species and source. All azole derivatives, except for fluconazole, showed good antifungal activity in vitro against *M. furfur* and *M. pachydermatis*

the possibility that M. pachydermatis develops resistance to azole-class antifungals has often been indicated in the literature based on in vitro tests, but only 2 cases have been reported in vivo

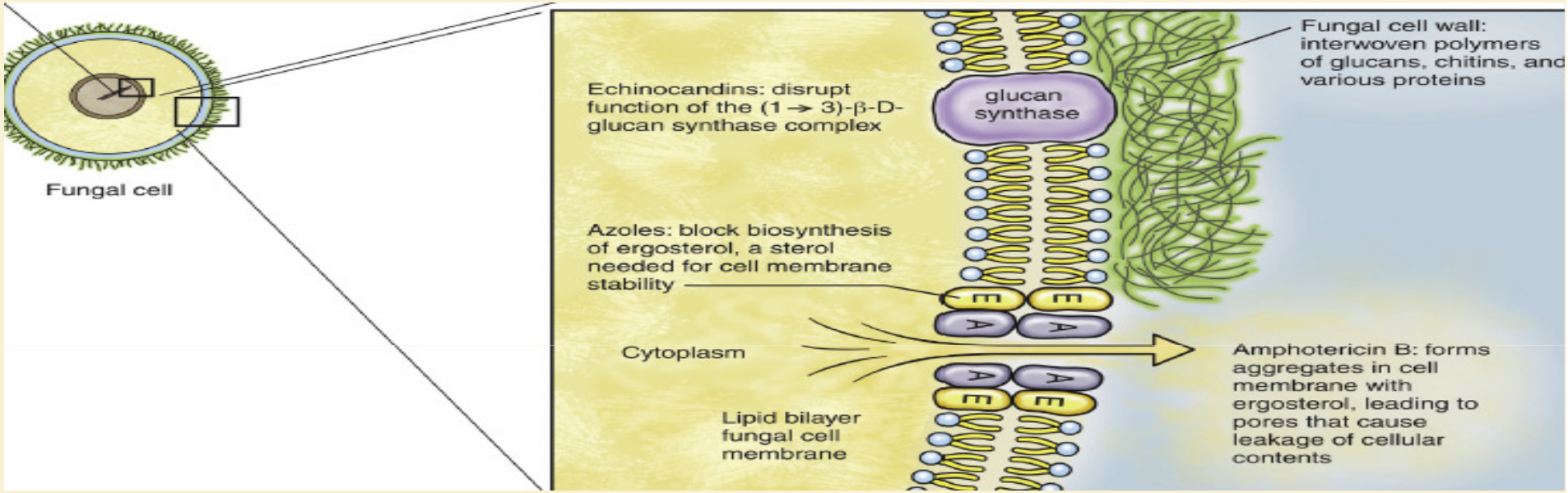
5-fluoropirimidine (Flucytosine)	DNA and RNA synthesis	-deficiency or lack of enzymes involved in the metabolism of flucytosine -regulation of the pyrimidine biosynthetic pathway

- **Flucytosine upon entry into the fungal cell is converted to 5-fluorouracil** (the metabolically active form), which inhibits DNA replication and protein synthesis.



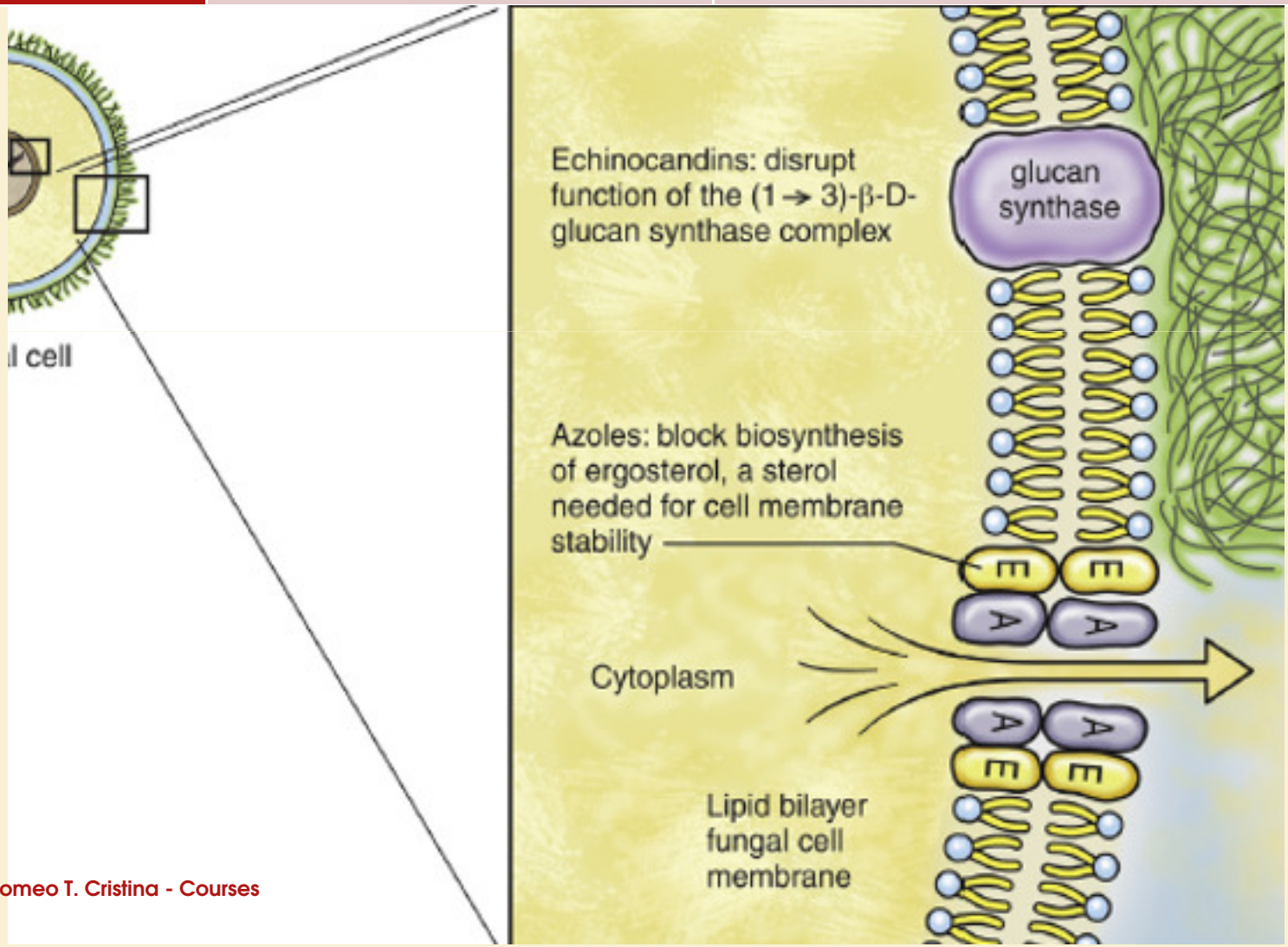
- The molecular mechanisms of flucytosine resistance are due to **mutations in the purine-cytosine permease enzyme (encoded by the FCY2 gene),** which is responsible for the degree of drug use in the cell.

Antifungal agent	Site of action	Mechanism of resistance
Polyenes (Amphotericin B, Nistatin)	inhibition of ergosterol biosynthesis	- absence of ergosterol (mutation to ERG3 or ERG6); -decreased ergosterol content in cells;



- Action: by **binding ergosterol from the fungal cell membrane** and forming a complex capable of modifying the cell membrane, with the formation of **membranous pores** leading to **loss of monovalent ions and then to cell death**.
- Resistance to isolates of *Candida spp., C. immitis, and Mucor spp.*

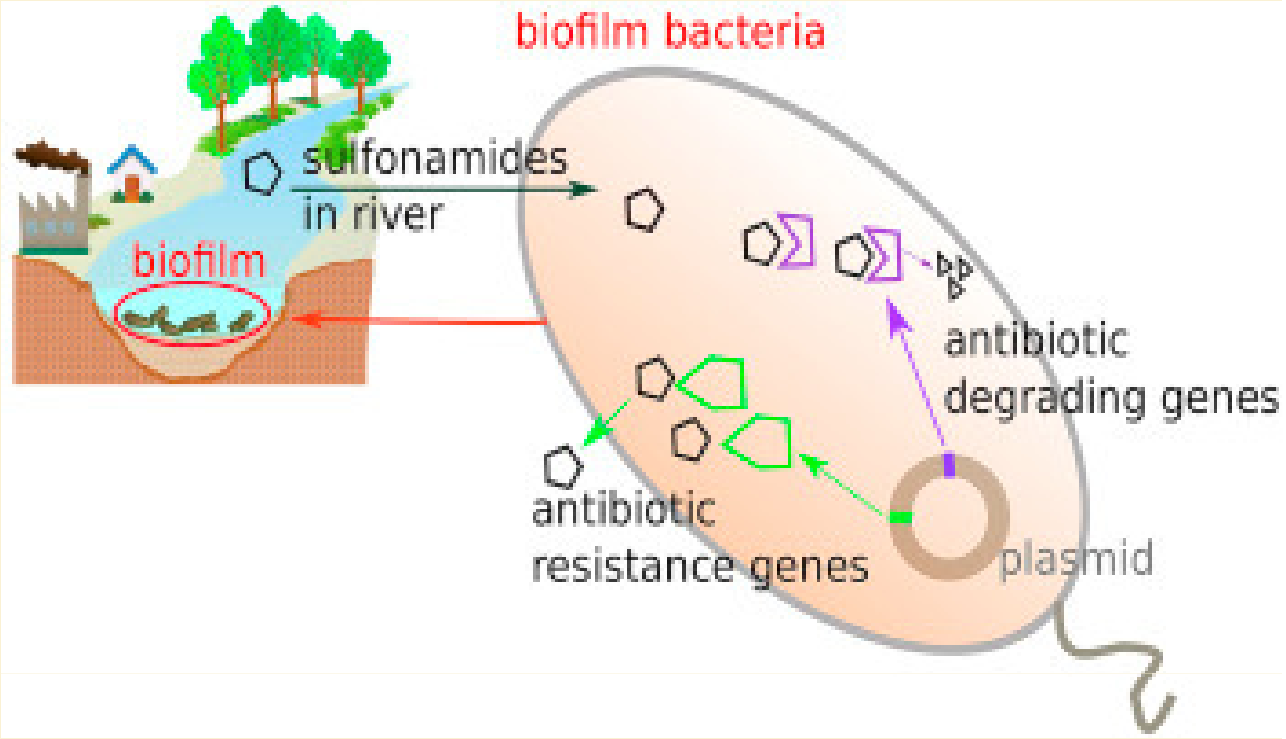
Antifungal agent	Site of action	Mechanism of resistance
Echinocandins (Caspofungins)	Inhibits β-1,3 glucan synthetase	- modification of the affinity of echinocandins for β (1,3) - glucan synthetase



Sulfonamide resistance

It can be:

- **Intrinsic**
- **Acquired**

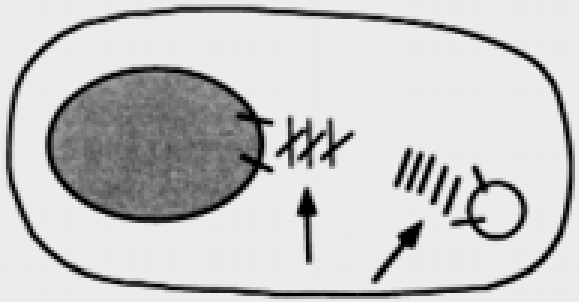


Intrinsic resistance: to bacteria and microorganisms that can take preformed folic acid from the environment that they cannot synthesize on their own.

Acquired resistance: it appears faster and lasts longer. It is based on the enzymatic adaptation of bacterial metabolism or of a species of bacteria or mediators of *factor R*.

Enzymatic alteration

By class of antimicrobial agent	Activity	Phenotypic determinant of resistance	Bacterial genera representative
Sulfonamides (Agents acting as antimetabolites) (It blocks folic acid metabolism by inhibiting dihydrofolate reductase)	Static	Sulfonamide-resistant dihydropteroate synthase, which can not be inhibited by sulfonamide.	*E. coli*
Trimethoprim (Agents acting as antimetabolites) (It blocks folic acid metabolism by inhibiting dihydrofolate reductase)	Static alone Cidal with sulfonamides	Group B drug-insensitive R67 dihydrofolate reductase, which can not be inhibited by trimethoprim	*E. coli*

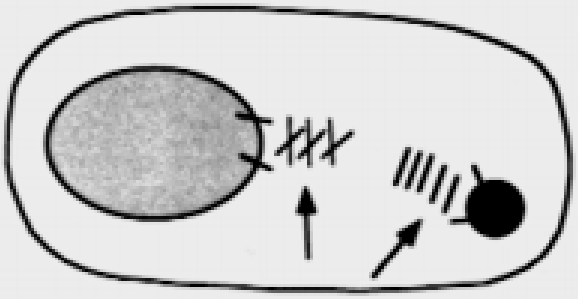


Sulfonamide

Drugresistant variations of the folate-forming enzyme dihydropteroate synthase (DHPS)

	MIC
E. coli	< 0.01 mM
E. coli (sul 1)	2.00 mM
E.coli (sul 2)	> 4.00 mM

Dihydrofolate reductase



Trimethoprim

Natural resistance determined by plasmids to sulfonamides (top) and trimethoprim (bottom)

EUROPEAN ANTIBIOTIC AWARENESS DAY

A EUROPEAN HEALTH INITIATIVE

Antibiotic resistance: synthesis of recommendations by expert policy groups

Alliance for the Prudent Use of Antibiotics

World Health Organization

EU guidelines against combinations for veterinary medicine (Sep 2015)

11.9.2015 EN Official Journal of the European Union C 299/7

COMMISSION NOTICE

Guidelines for the prudent use of antimicrobials in veterinary medicine

(2015/C 299/04)

Responsible use of antimicrobials: General principles

- **Anti-infective a.u.v. are important drugs in the prevention and treatment of bacterial, viral, fungal and parasitic diseases being essential for maintaining the health and welfare of the animals.**
- **The main objectives of the use of anti-infective drugs in farm animals:**

- Protection of animal and public health.
- Responsible use and optimization of the effectiveness of each relevant antibiotic/disease, now and in the future.
- Permanent training of stakeholders: farmers, professionals in the field

- The **administration of antibiotics** should be **complementary to good management practices**, as many disease states can be avoided or minimized by:

• use of management practices that significantly reduce exposure to disease-generating bacteria;

• optimizing the environment for the animal, including good hygiene, nutrition, and consistent vaccination programs.

- **Animal health = the result of a wide range of factors grouped into:**

1. **Factors related to the animal**
2. **The growth system**
3. **Management**

- These factors will be evaluated separately, but also in correlation, from their interdependence, most often resulting in the complexity of clinical situations.
- **So, every situation can have its specific therapeutic!**

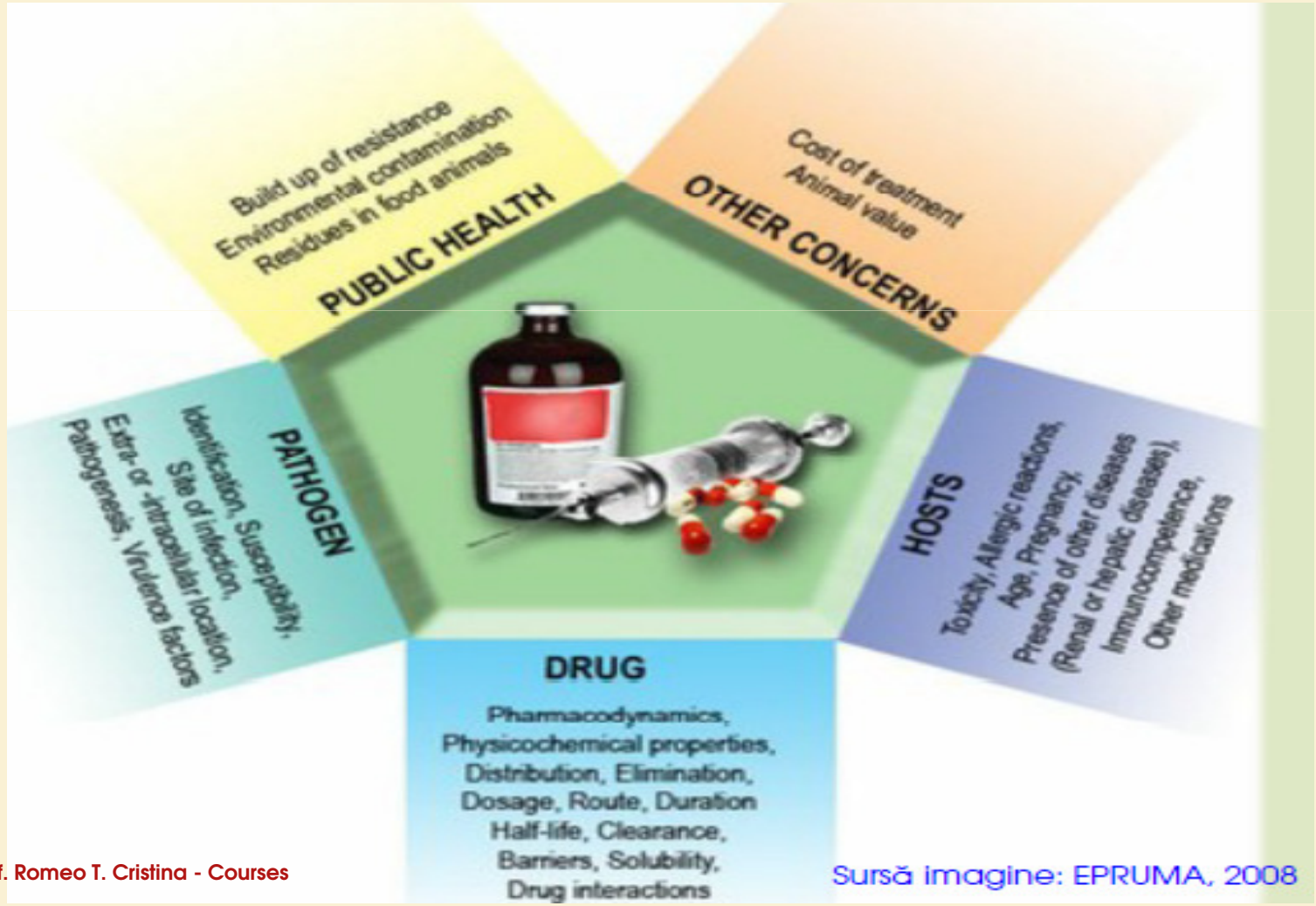
The professional attitude should focus on maintaining the effectiveness of antimicrobial agents and include: 112

a. Accumulation of information on disease prevention, management, and reduction strategies;
b. Evaluation of the ability of antimicrobial agents to select, in animals, resistant microorganisms and the relative importance of this resistance for public health and animal health;
c. Compliance with the recommendations regarding the responsible use of antimicrobial agents, in the breeding of animals, under the provisions of the marketing authorizations;
d. Proper storage of anti-infectives and appropriate methods of disposal of products

The responsibility of the veterinarian is to:

- a. perform a proper clinical examination
- b. administer/prescribe antimicrobial agents only when necessary, taking into account the OIE list of important antimicrobial agents in MV;
- c. choose antimicrobial agents, based on clinical experience and, where possible, diagnostic information provided by a laboratory (eg pathogen isolation/identification, antibiogram);
- d. ensures a complete treatment protocol, including precautionary measures, waiting times, especially when extra-label or off-label is prescribed.

Criteria for choosing antimicrobial agents



Sursă imagine: EPRUMA, 2008

The integrated solution:

a) **Efficient control of antibioresistance** by developing modern kits for microbiological and molecular analyzes on specific genes most commonly encountered.

b) **Realization of virtual databases** (of type gene bank), for the identified species.

c) **Designing new therapeutic formulas**, with antimicrobial efficiency against a wide range of microorganisms.

d) **The early detection of antibioresistance**, with the identification of risk factors, will lead to the rational and safe use of anti-infectious agents in humans and animals.

e) **Constant monitoring of the phenomenon of resistance** will lead to an increase in the quality of life, by adapting to the realities encountered, following the use of the methodologies envisaged.

Thank you for your attention!