Introduction to Microbiology

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Antimicrobial resistance

Overview

Topics that will be discussed this lecture are:

- ***Antimicrobial resistance mechanisms of bacteria***
- ***Antimicrobial resistance, the situation globally***

Ref:

An overview of the antimicrobial resistance mechanisms of bacteria

2021 AWaRe classification

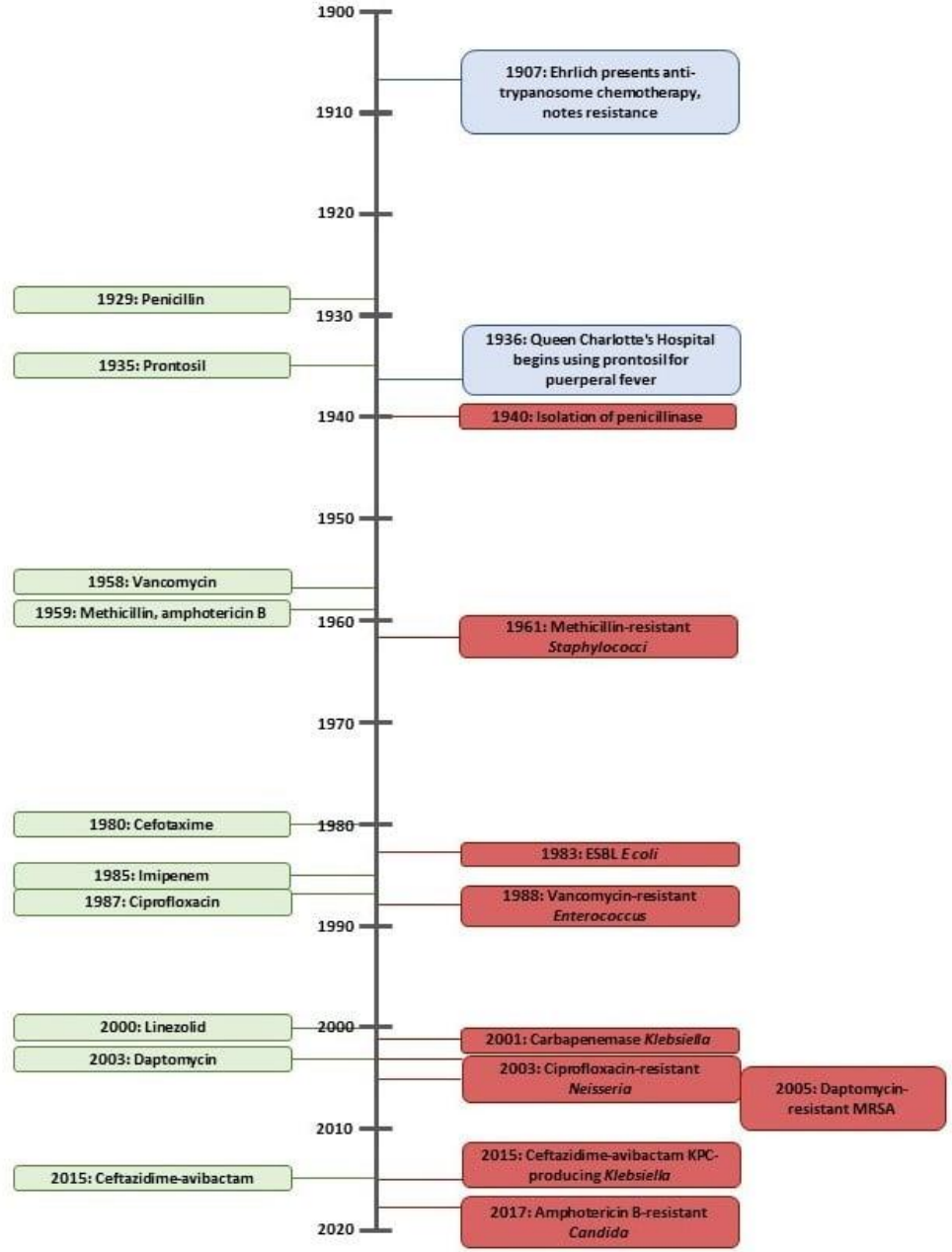
Antimicrobial resistance mechanisms of bacteria

- The first antibiotics were derived from the evolutionary arms race between microbes and their ecological competitors (fellow microbes, fungi, plants, and animals), and, as a result, the emergence of resistance **is entirely predictable**.

- Most pathogenic microorganisms have the capability of developing resistance to at least some antimicrobial agents

- The advent of antimicrobial resistance has added significantly to the impact of infectious diseases, in number of infections, as well as added healthcare costs.

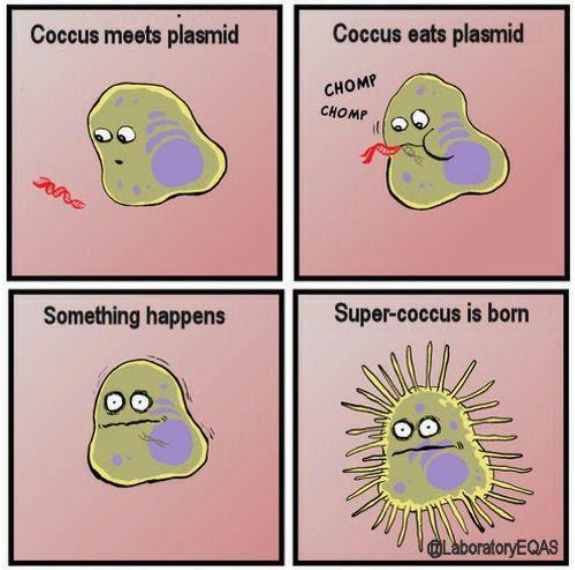
- Antimicrobial agents can be divided into groups based on the mechanism of antimicrobial activity.

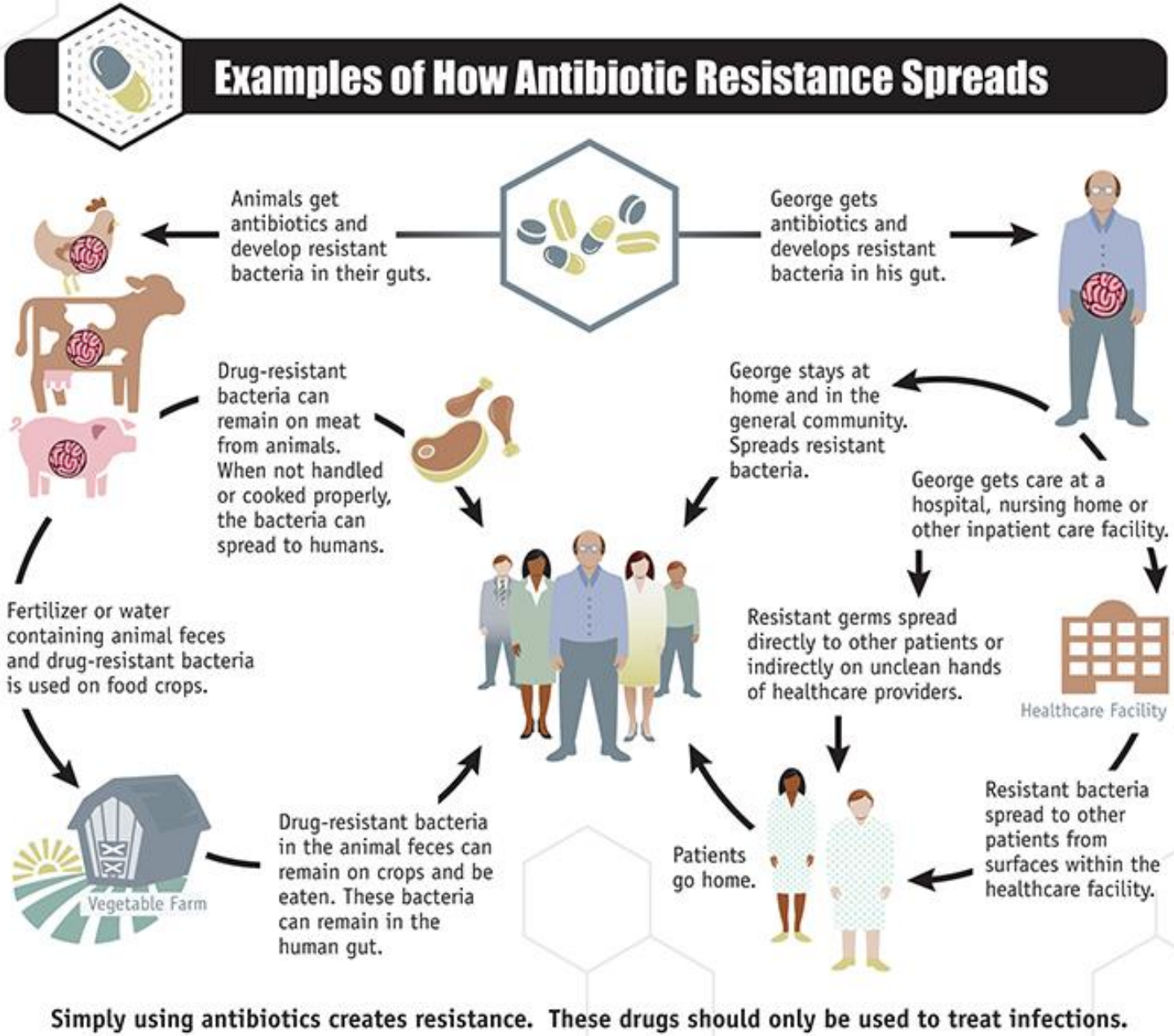

1900
1907: Ehrlich presents anti-trypanosome chemotherapy, notes resistance
1910
1920
1929: Penicillin
1930
1936: Queen Charlotte's Hospital begins using prontosil for puerperal fever
1935: Prontosil
1940
1940: Isolation of penicillinase
1950
1958: Vancomycin
1959: Methicillin, amphotericin B
1960
1961: Methicillin-resistant *Staphylococci*
1970
1980: Cefotaxime
1980
1983: ESBL *E coli*
1985: Imipenem
1987: Ciprofloxacin
1988: Vancomycin-resistant *Enterococcus*
1990
2000: Linezolid
2000
2001: Carbapenemase *Klebsiella*
2003: Daptomycin
2003: Ciprofloxacin-resistant *Neisseria*
2005: Daptomycin-resistant MRSA
2010
2015: Ceftazidime-avibactam
2015: Ceftazidime-avibactam KPC-producing *Klebsiella*
2017: Amphotericin B-resistant *Candida*
2020
Antimicrobial discovery or implementation
Resistance identified
Historic events in antimicrobial use and development

Antimicrobial resistance mechanisms of bacteria

Mechanism of Action	Antimicrobial Groups
Inhibit Cell Wall Synthesis	β-Lactams
	Carbapenems
	Cephalosporins
	Monobactams
	Penicillins
	Glycopeptides
Depolarize Cell Membrane	Lipopeptides
Inhibit Protein Synthesis	Bind to 30S Ribosomal Subunit
	Aminoglycosides
	Tetracyclines
	Bind to 50S Ribosomal Subunit
	Chloramphenicol
	Lincosamides
	Macrolides
	Oxazolidinones
	Streptogramins
Inhibit Nucleic Acid Synthesis	Quinolones
	Fluoroquinolones
Inhibit Metabolic Pathways	Sulfonamides
	Trimethoprim

- [Emergence and spread of antibiotic-resistant bacteria]





Antimicrobial resistance mechanisms of bacteria- Origins of resistance

- Levels of resistance may vary greatly within related bacterial groups.
- **Susceptibility** and **resistance** are usually measured as a function of **minimum inhibitory concentration (MIC),** the minimal concentration of drug that will inhibit growth of the bacteria.
- If that **average MIC for a species is in the resistant part of the range**, the species is considered to have **intrinsic resistance** to that drug.
- Bacteria may also **acquire resistance genes** from other related organisms, and the level of resistance will vary depending on the species and the genes acquired.

Antimicrobial resistance mechanisms of bacteria- Natural resistance

- **Intrinsic resistance** may be defined as a trait that is shared universally within a bacterial species, is **independent of previous antibiotic exposure**, and **not related to horizontal gene transfer**.

- The most common bacterial mechanisms involved in intrinsic resistance are **reduced permeability of the outer membrane** (most specifically the lipopolysaccharide, LPS, in gram negative bacteria) and **the natural activity of efflux pumps**.

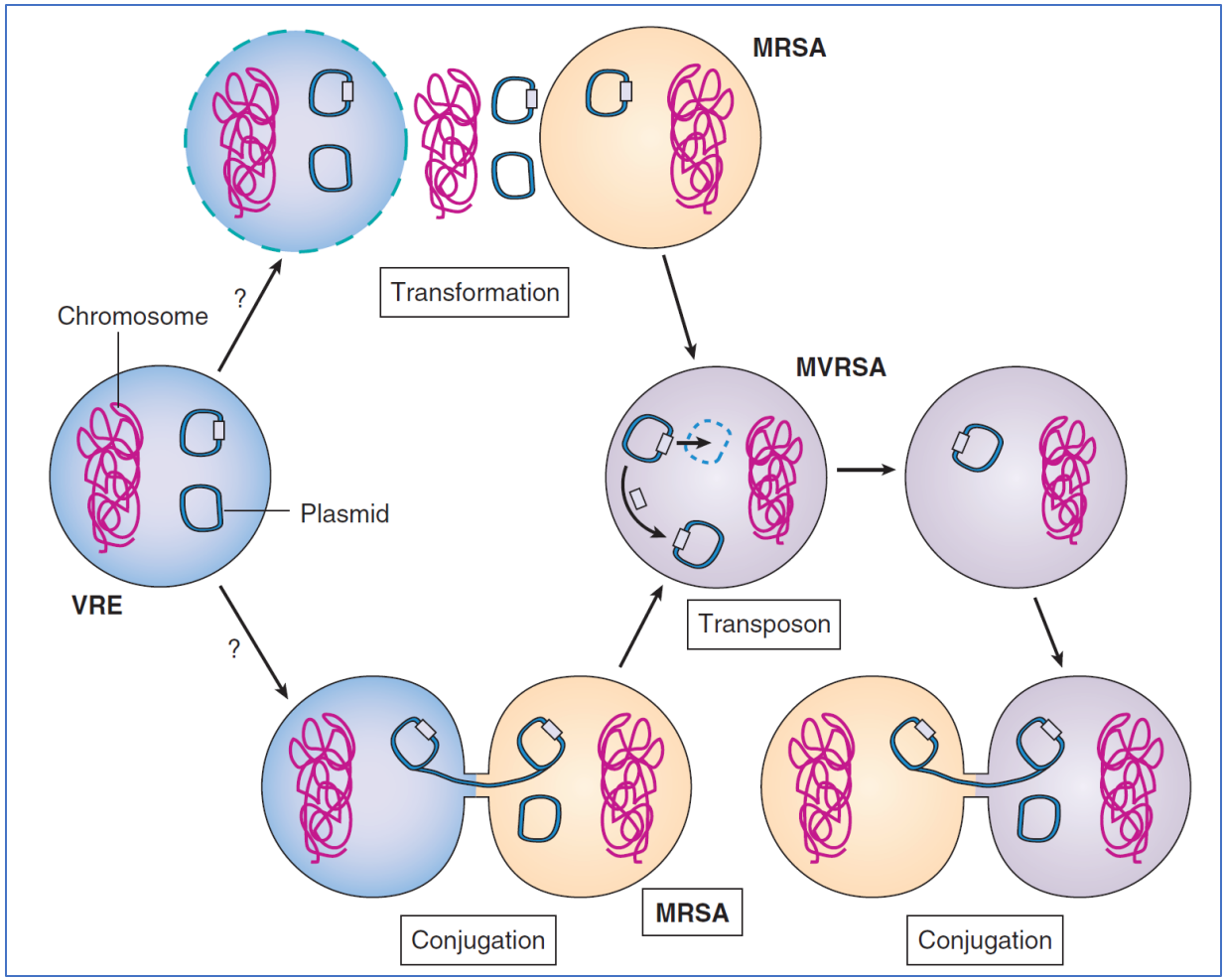
Antimicrobial resistance mechanisms of bacteria- Acquired resistance

Acquisition of genetic material that confers resistance is possible through:

- Transformation, transposition, and conjugation (all termed **horizontal gene transfer**—HGT); plus, **the bacteria may experience mutations to its own chromosomal DNA**

- The acquisition may be temporary or permanent.

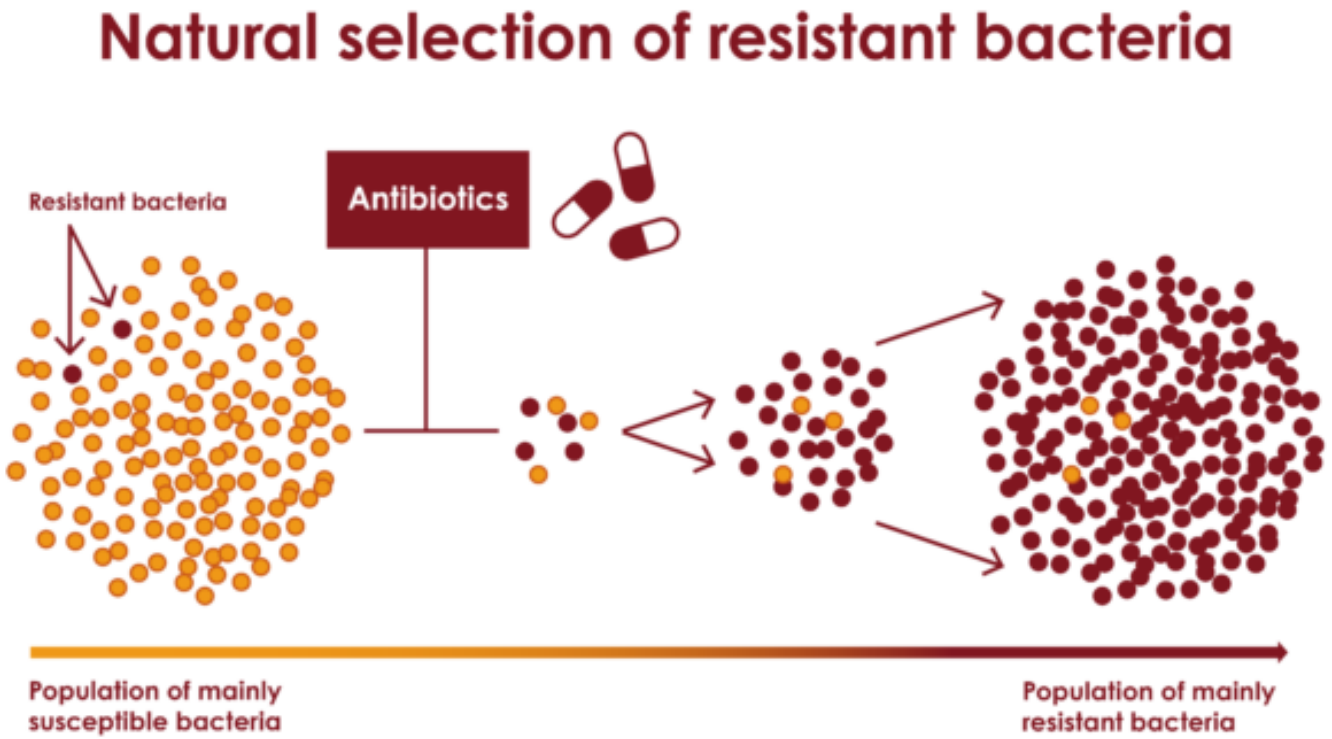
- **Plasmid-mediated transmission of resistance genes is the most common route** for acquisition of outside genetic material



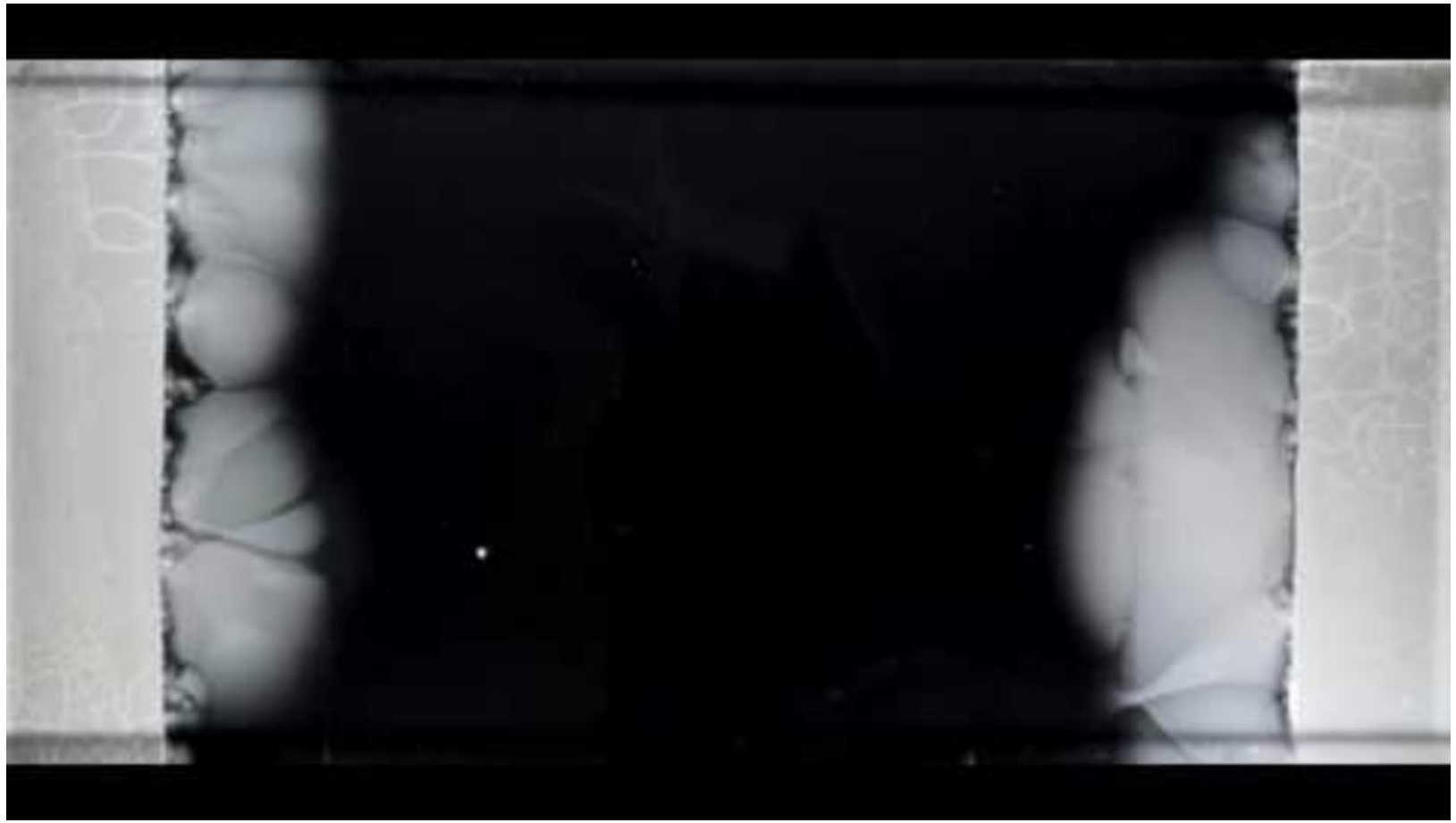
Antimicrobial resistance mechanisms of bacteria- Acquired resistance

- Bacteria have an average mutation rate of 1 for every 10^6 to 10^9 cell divisions, and most of these mutations will be deleterious to the cell.

- Mutations that aid in antimicrobial resistance usually only occur in a few types of genes; those encoding **drug targets**, those encoding **drug transporters**, those encoding **regulators that control drug transporters**, and those encoding **antibiotic-modifying enzymes**

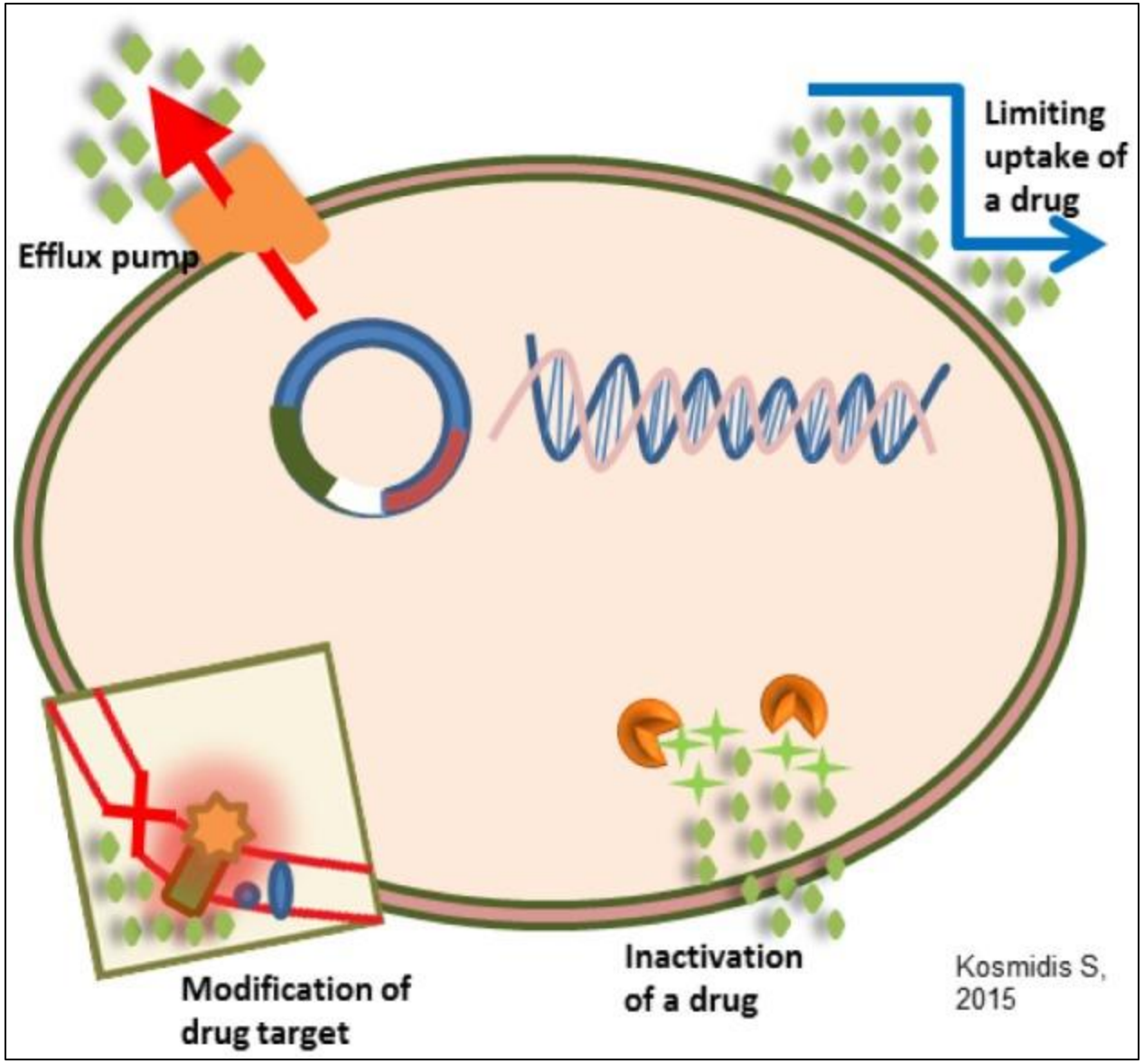


A cinematic approach to drug resistance



Antimicrobial resistance mechanisms of bacteria- Mechanisms of resistance

- Antimicrobial resistance mechanisms fall into four main categories:
- (1) limiting uptake of a drug;
- (2) modifying a drug target;
- (3) inactivating a drug;
- (4) active drug efflux.



[Mechanisms of antibiotic resistance]

Osmosis from Elsevier

Antimicrobial resistance mechanisms of bacteria- Limiting drug uptake

- The structure and functions of the **LPS layer in gram negative bacteria** provides a barrier to certain types of molecules. This gives those bacteria innate resistance to certain groups of large antimicrobial agents

- The mycobacteria have an outer membrane that has a **high lipid content**, and so hydrophobic drugs such as rifampicin and the fluoroquinolones have an easier access to the cell, but hydrophilic drugs have limited access.

- Bacteria that **lack a cell wall**, such as Mycoplasma and related species, are therefore intrinsically resistant to all drugs that target the cell wall including β-lactams and glycopeptides.

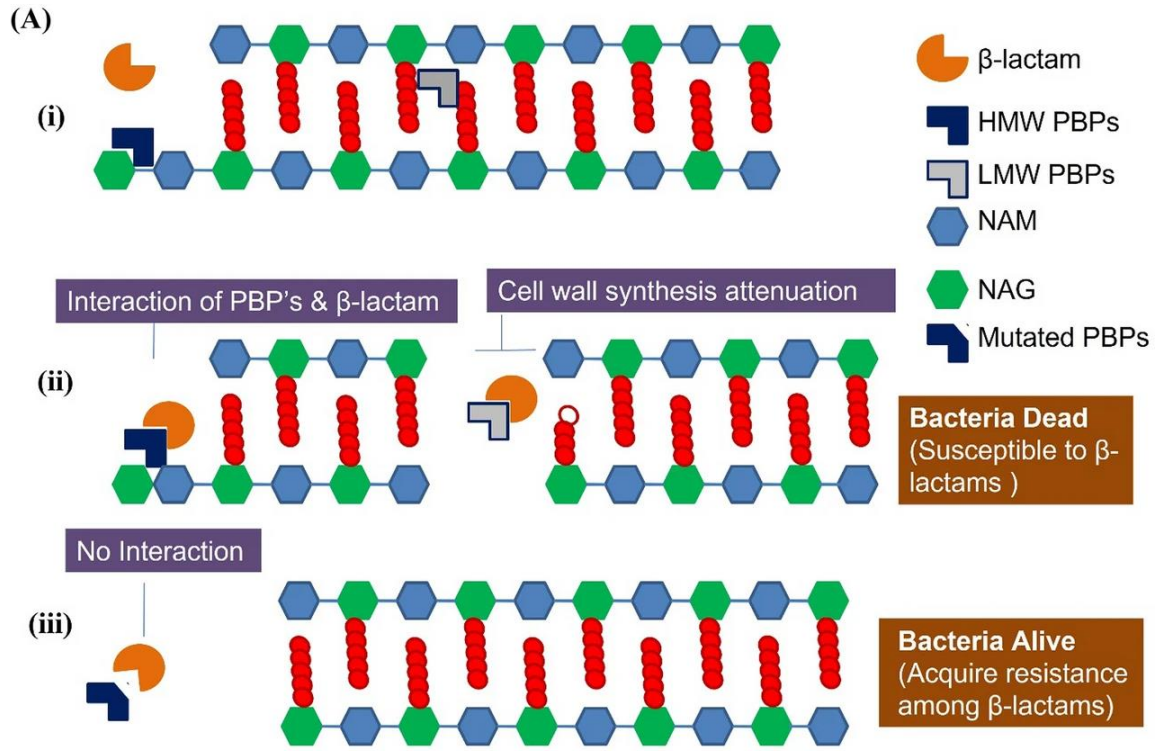
- The thick, sticky consistency of the **biofilm matrix** which contains polysaccharides, and proteins and DNA from the resident bacteria, makes it difficult for antimicrobial agents to reach the bacteria. Thus, to be effective, much higher concentrations of the drugs are necessary.

Antimicrobial resistance mechanisms of bacteria- Modification of drug targets

- One mechanism of resistance to the β-lactam drugs used almost exclusively by gram-positive bacteria is via **alterations in the structure and/or number of PBPs (penicillin-binding proteins).**

- Resistance to drugs that target the **ribosomal subunits may occur via ribosomal mutation or ribosomal subunit methylation**. These mechanisms interfere with the ability of the drug to bind to the ribosome.

- For drugs that target nucleic acid synthesis, resistance is via **modifications in DNA gyrase or topoisomerase IV**



Antimicrobial resistance mechanisms of bacteria- Drug inactivation

- There are two main ways in which bacteria inactivate drugs:
  Degradation of the drug, or by transfer of a chemical group to the drug. The β-lactamases are a very large group of drug hydrolyzing enzymes.

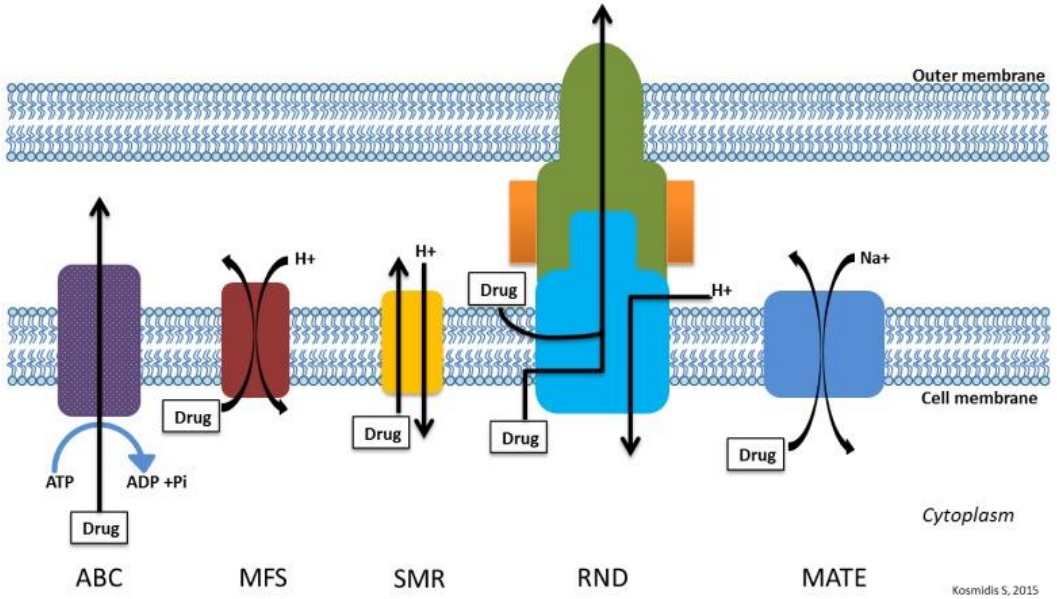
- The β-lactamases (originally called penicillinases and cephalosporinases) inactivate β-lactam drugs by **hydrolyzing a specific site in the β-lactam ring structure**, causing the ring to open. The open-ring drugs are not able to bind to their target PBP proteins.

- The production of β-lactamases is **the most common resistance mechanism used by gram negative bacteri**a against β-lactam drugs, and the **most important resistance mechanism against penicillin and cephalosporin drugs**

Antimicrobial resistance mechanisms of bacteria- Drug efflux

- Bacteria possess **chromosomally encoded genes for efflux pumps**. Some are **expressed constitutively**, and **others are induced or overexpressed** (high-level resistance is usually via a mutation that modifies the transport channel) under certain environmental stimuli or when a suitable substrate is present.

- **The efflux pumps function primarily to rid the bacterial cell of toxic substances**, and many of these pumps will transport a large variety of compounds (multi-drug [MDR] efflux pumps).



Antimicrobial resistance mechanisms of bacteria- the β-lactam drugs as an example.

- The most widely used group of antimicrobial agents are the β-lactam drugs. The members of this drug group all share a specific core structure which consists of a four-sided β-lactam ring.

- Resistance to the β-lactam drugs occurs through three general mechanisms:

(1) preventing the interaction between the target PBP and the drug, usually by modifying the ability of the drug to bind to the PBP (this is mediated by alterations to existing PBPs or acquisition of other PBPs;

(2) the presence of efflux pumps that can extrude β-lactam drugs;

(3) hydrolysis of the drug by β-lactamase enzymes

Beta-lactamase inhibitors are another type of antibiotic that are co-administered with beta-lactam antibiotics, to prevent bacteria from disabling these antibiotics using their enzymes

Table 3. Antimicrobial resistance mechanisms.

Drug	Drug Uptake Limitation	Drug Target Modification	Drug Inactivation	Efflux Pumps
β-Lactams	Decreased numbers of porins, no outer cell wall	Gram pos—alterations in PBPs	Gram pos, gram neg—β-lactamases	RND
Carbapenems	Changed selectivity of porin			
Cephalosporins	Changed selectivity of porin			
Monobactams				
Penicillins				
Glycopeptides	Thickened cell wall, no outer cell wall	Modified peptidoglycan		
Lipopeptides		Modified net cell surface charge		
Aminoglycosides	Cell wall polarity	Ribosomal mutation, methylation	Aminoglycoside modifying enzymes, acetylation, phosphorylation, adenylation	RND
Tetracyclines	Decreased numbers of porins	Ribosomal protection	Antibiotic modification, oxidation	MFS, RND
Chloramphenicol		Ribosomal methylation	Acetylation of drug	MFS, RND
Lincosamides		Gram pos—ribosomal methylation		ABC, RND
Macrolides		Ribosomal mutation, methylation		ABC, MFS, RND
Oxazolidinones		Ribosomal methylation		RND
Streptogramins				ABC
Fluoroquinolones		Gram neg—DNA gyrase modification	Acetylation of drug	MATE, MFS, RND
		Gram pos—topoisomerase IV		
Sulfonamides		DHPS reduced binding, overproduction of resistant DHPS		RND
Trimethoprim		DHFR reduced binding, overproduction of DHFR		RND

ABC—ATP binding cassette family, DHFR—dihydrofolate reductase, DHPS—dihydropteroate synthase, MATE—multidrug and toxic compound extrusion family, MFS—major facilitator superfamily, PBP—penicillin-binding protein, RND—resistance-nodulation-cell division family.

What is the role of efflux pumps in bacterial resistance?

- A. Enzymatically degrade antibiotics
- B. Prevent antibiotic entry into the cell
- C. Actively transport antibiotics out of the cell
- D. Modify antibiotic binding sites

What is the primary mechanism of resistance to fluoroquinolones in bacteria?

- A. Production of efflux pumps
- B. Modification of topoisomerase and DNA gyrase enzymes
- C. Enzymatic inactivation of the antibiotic
- D. Alteration of the 30S ribosomal subunit

A young child presents with meningitis caused by *Streptococcus pneumoniae*. The strain is resistant to penicillin. What is the most likely resistance mechanism?

- A. Alteration of the 30S ribosomal subunit
- B. Beta-lactamase production
- C. Modification of penicillin-binding proteins
- D. Decreased membrane permeability

Which of the following strategies is most effective in reducing the development of antimicrobial resistance?

- A. Using broad-spectrum antibiotics for all infections
- B. Encouraging over-the-counter antibiotic access
- C. Completing prescribed antibiotic courses and limiting unnecessary use
- D. Relying solely on vaccines to control bacterial infections

Some important acronyms in antimicrobial resistance

- Antimicrobial resistance **(AMR)**
- Multidrug resistant **(MDR)**
- Extensively drug-resistant **(XDR)**
- **ESKAPE** is an acronym comprising the scientific names of six **highly virulent** and **antibiotic-resistant** bacterial pathogens including Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter spp. The acronym is sometimes extended to ESKAPEE to include Escherichia coli.

Some important acronyms in antimicrobial resistance

- **Extended spectrum β-lactamase** (ESBL) are enzymes that confer resistance to most beta-lactam antibiotics, including **penicillins**, **cephalosporins**, and the **monobactam** aztreonam. Infections with ESBL-producing organisms have been associated with **poor outcomes**.

- The gastrointestinal tract is the main reservoir for ESBL-producing Enterobacteriaceae, and colonization with such organisms is a strong risk factor for subsequent infection with them. Most clinical factors associated with colonization and infection with ESBL-producing organisms involve healthcare exposure, such as hospitalization, residence in a long-term care facility, hemodialysis use, and presence of an intravascular catheter.

- The preferred, proven therapeutic options for severe infections caused by extended-spectrum beta-lactamase (ESBL)-producing organisms are carbapenems (imipenem, meropenem, and ertapenem).

Which of the following bacteria is commonly associated with extended-spectrum beta-lactamase (ESBL) production?

- A. *Escherichia coli*
- B. *Streptococcus pneumoniae*
- C. *Mycobacterium tuberculosis*
- D. *Clostridium difficile*

Which of the following is NOT part of the ESKAPEE group of pathogens?

- A. *Enterococcus faecium*
- B. *Escherichia coli*
- C. *Klebsiella pneumoniae*
- D. *Salmonella Typhi*

The situation globally

- Antimicrobial resistance (AMR) is a global threat that claims 700 000 lives every year. If no urgent actions are taken, by 2050, AMR will cause an estimated loss of 10 million lives and $US100 trillion. *

- Bacteria showing resistance to at least three different classes of antimicrobials, defined as **multidrug resistant (MDR),** have become common, especially in hospitals; there is a risk of entering a so-called **"post-antibiotic era"** in a few years, in which infections apparently under control easily turn into lethal threats. **

- AMR leads to **longer hospital stays**, **higher medical costs** and **increased mortality**. Antibiotic resistance **occurs naturally**, but misuse of antibiotics in humans and animals is accelerating the process. Tackling antibiotic resistance is a high priority for **the World Health Organization (WHO)**.

The situation globally

- WHO is coordinating a global campaign to **raise awareness** and encourage best practices among the public, policymakers, health and agriculture professionals

- WHO published its first ever list of antibiotic-resistant "priority pathogens" – in 2017 a catalogue of **12 families of bacteria that pose the greatest threat to human health.**

- The list was drawn up in a **bid to guide and promote research and development (R&D)** of new antibiotics, as part of WHO's efforts to address growing global resistance to antimicrobial medicines.

The situation globally

WHO priority pathogens list for R&D of new antibiotics

Priority 1: CRITICAL

- *Acinetobacter baumannii*, carbapenem-resistant
- *Pseudomonas aeruginosa*, carbapenem-resistant
- *Enterobacteriaceae*, carbapenem-resistant, ESBL-producing

Priority 2: HIGH

- *Enterococcus faecium*, vancomycin-resistant
- *Staphylococcus aureus*, methicillin-resistant, vancomycin-intermediate and resistant
- *Helicobacter pylori*, clarithromycin-resistant
- *Campylobacter* spp., fluoroquinolone-resistant
- *Salmonellae*, fluoroquinolone-resistant
- *Neisseria gonorrhoeae*, cephalosporin-resistant, fluoroquinolone-resistant

Priority 3: MEDIUM

- *Streptococcus pneumoniae*, penicillin-non-susceptible
- *Haemophilus influenzae*, ampicillin-resistant
- *Shigella* spp., fluoroquinolone-resistant

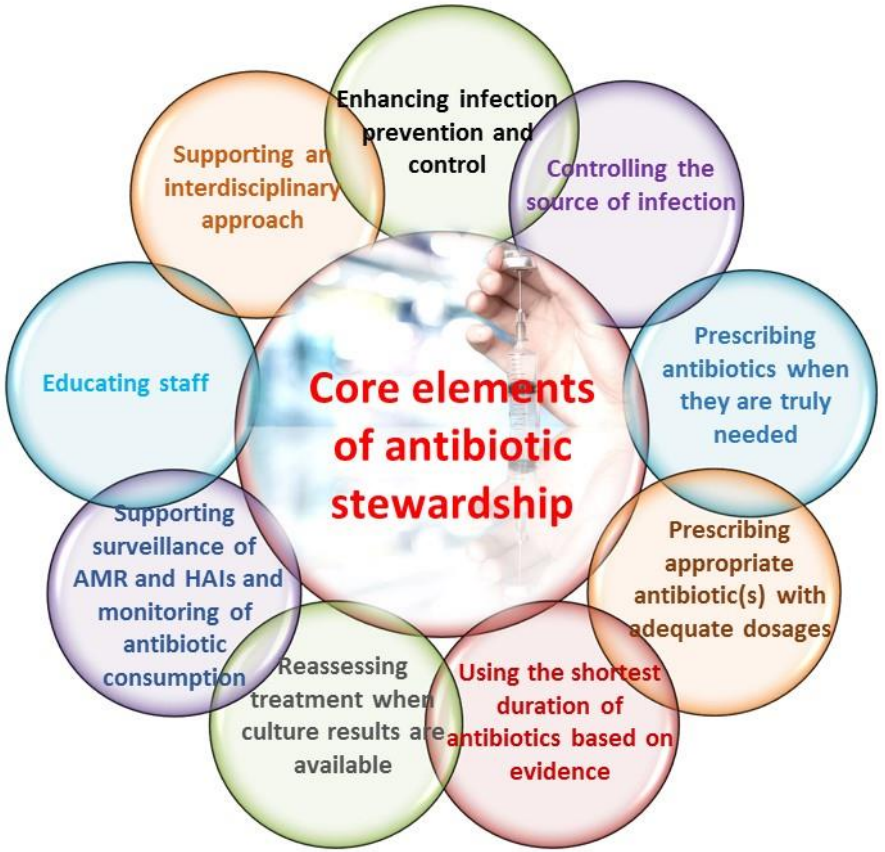
The situation globally

- The **AWaRe Classification** of antibiotics was developed in 2017 by the WHO Expert Committee on Selection and Use of Essential Medicines as a tool to support **antibiotic stewardship** efforts at local, national and global levels,

- Antibiotics are classified into three groups, **Access, Watch and Reserve**, taking into account the impact of different antibiotics and antibiotic classes on antimicrobial resistance, to emphasize the importance of their appropriate use.

- **Antibiotic stewardship** is the effort to measure and improve how antibiotics are prescribed by clinicians and used by patients. Improving antibiotic prescribing and use is critical to effectively treat infections, protect patients from harms caused by unnecessary antibiotic use, and combat antibiotic resistance.

The situation globally



The situation in low- and middle-income countries

- AMR affects all countries, but the burden is disproportionately higher in low-income and middle-income countries (LMIC). * Why ?

The situation in low- and middle-income countries

- Economic factors:

1. LMIC Lack resources (functional and infrastructural) for healthcare to reach a large population, more so in rural areas.

2. Restricted access to qualified healthcare workers.

3. Often unqualified and profit-driven providers sell antimicrobials over the counter (OTC) for mild to moderate illnesses, a large proportion of which are self-limiting viral infections.

> *The reality is that governments will sooner or later bear the cost of AMR: they can either do so proactively by taking action now and pay less fo better outcomes, or remain unprepared and end up spending much more taxpayer money on far worse outcomes further down the line.*

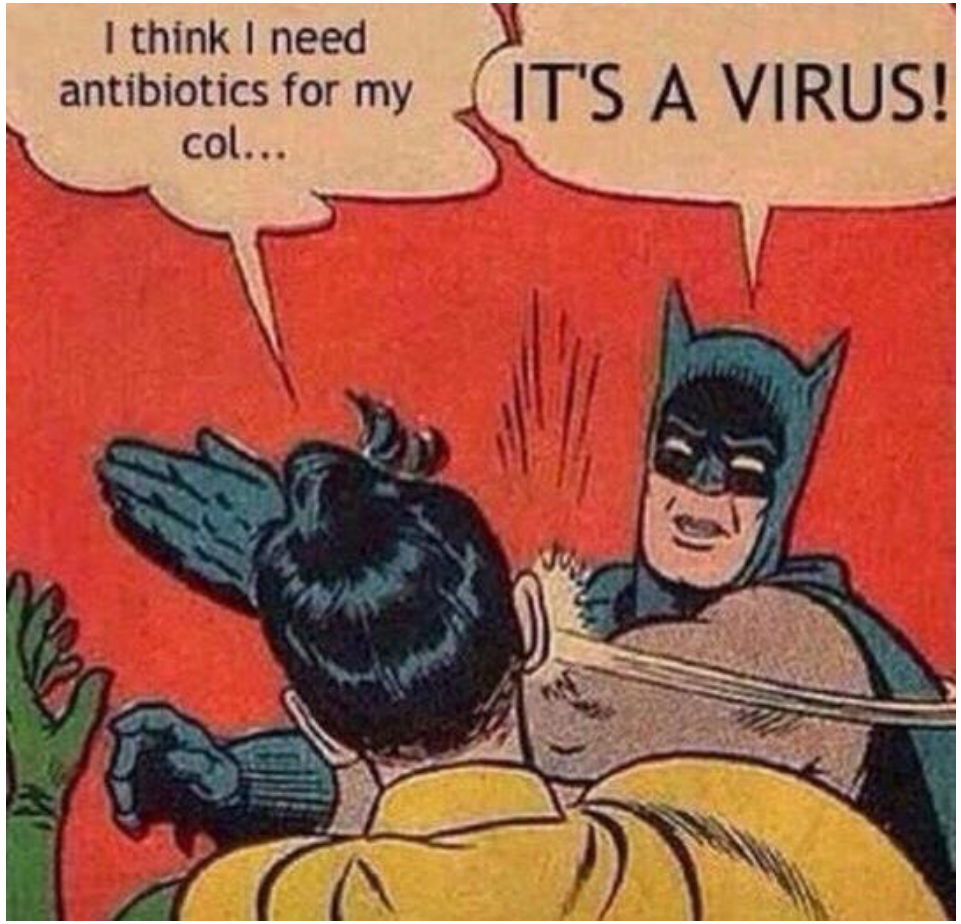
The situation in low- and middle-income countries

- Sociological factors:

1. Poor educational status and low awareness leave populations with popular myths, cultural practices and belief systems towards the use of medicines, especially antibiotics (as a quick fix).

2. Medicines obtained from traditional practitioners are often unknown chemical agents mixed with antimicrobials in substandard doses.

3. Driven by the desire to get well soon and at minimal cost (again, a quick fix), patients often demand treatment regardless of the type of infection (bacterial or viral) and avoid necessary investigations during consultations.



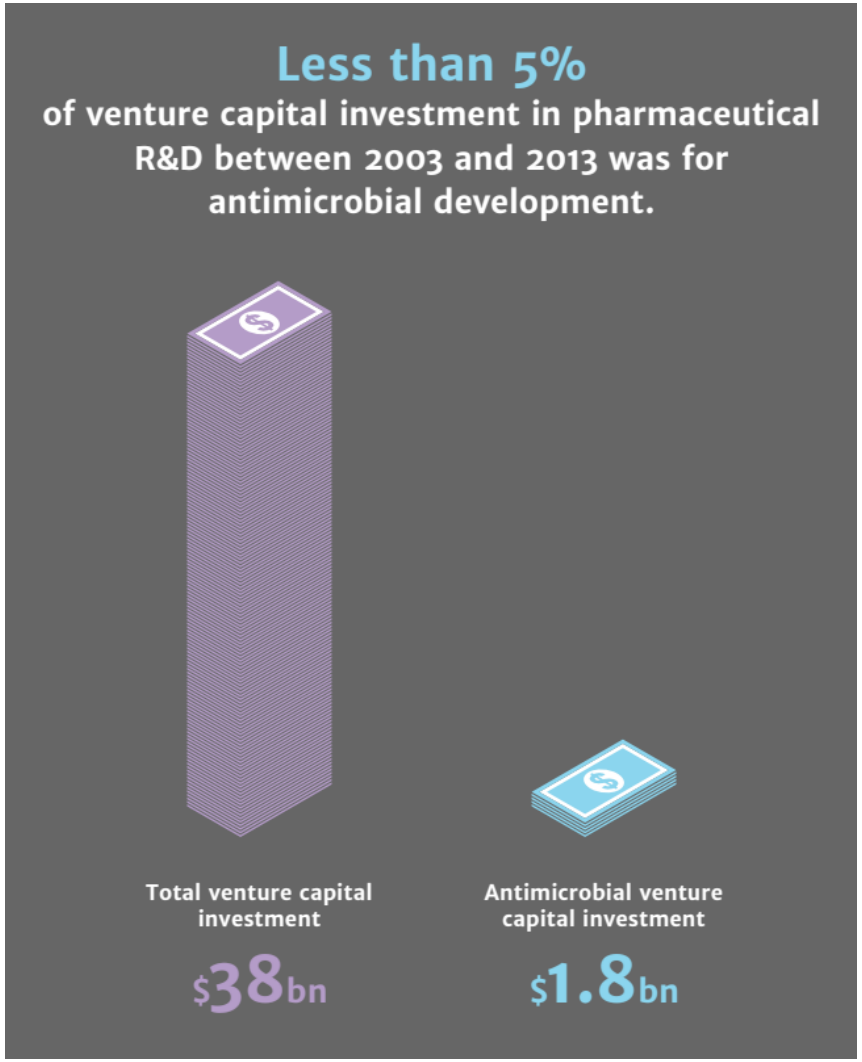
The situation in low- and middle-income countries

- Industry factors:

1. This perception that infectious disease is somehow ‘yesterday’s problem’ has led to an over-adjustment in terms of research priorities in favour of non-communicable diseases, and ultimately a neglect of R&D

2. With the diminished production of newer antibiotics and growing AMR, remaining antibiotics have become extremely expensive and are unaffordable in many low-income and middle-income countries.

3. Pharmaceutical companies’ incentives to medical practitioners and drug dispensers to prescribe specific antimicrobials further escalate the use and cost of antimicrobials.



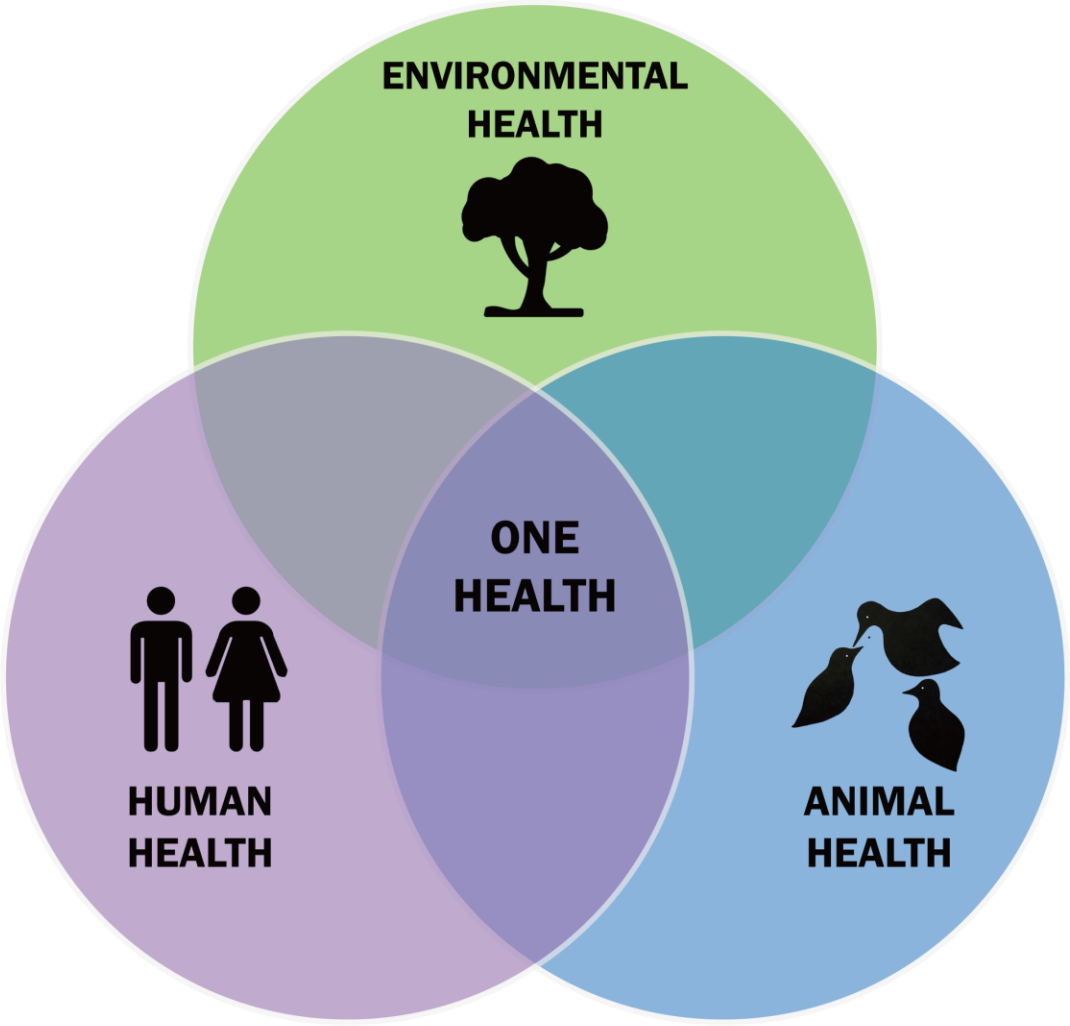
The situation in low- and middle-income countries

- <u>Ecological factors:</u>

1. AMR cannot be tackled well without an ecological approach embedded in the concept of ‘One Health’.

2. Around 70% of medically important antimicrobials in the the USA are sold for use in food-producing animals. Such widespread antimicrobial use also echoes across Europe.

3. Although the available information from many low-income and middle-income countries is limited, empirical estimates suggest that the antimicrobial use in animal food is very high.



The situation in low- and middle-income countries

- Technological factors:

1. Technological innovations in diagnostics to rapidly detect infections and AMR are critical for both improved patient care and better surveillance.

2. Diagnostics to inform the appropriate prescription of antimicrobials are not available at the point of care, while antimicrobials are easily accessible OTC and a wide variety of infections are treated empirically.

3. Healthcare innovations through computerised real-time reporting of data are essential for improved surveillance and action.

Tackling antimicobial resistance

The situation in Jordan

Int J Occup Med Environ Health. 2007;20(4):373-80. doi: 10.2478/v10001-007-0038-9.

Self-medication with antibiotics in Jordanian population

Sayer I Al-Azzam [1], Belal A Al-Husein, Firas Alzoubi, Majed M Masadeh, Mohammad Ali S Al-Horani

- 842 (39.5%) of 2133 antibiotic users identified via the survey had used antibiotics without a prescription within a one-month study period.

The prevalence and patterns of self-medication with antibiotics in Jordan: A community-based study

Mohammad B. Nusair[1] | Sayer Al-azzam[2] | Hamza Alhamad[3] | Mohammad Y. Momani[1]

- 40.4% reported having used an antibiotic without a prescription in the previous month.

The situation in Jordan

- We need more data!
- We need social change!
- We need more money to reach RnD!

The situation in Jordan

- Proposal to adapt **AWaRe Categorization for the Antibiotics registered in Jordan**. Rational Drug Use and Pharmacovigilance Department. December - 2020 .

1. Reduction of the evolution of antimicrobial resistance.
2. Synchronization with published clinical practice guidelines for the management of common and/ or serious infections.
3. Integration of cost parameters.
4. Encouragement of responsible prescription practices among physicians and dispensing among pharmacists.
5. Assignment of multi-level prescription responsibility.

Further reading and material:

- Antibiotic stewardship
- https://www.cdc.gov/antibiotic-use/core-elements/index.html#:~:text=Antibiotic%20stewardship%20is%20the%20effort,use%2C%20and%20combat%20antibiotic%20resistance.

- https://www.who.int/en/news-room/detail/27-02-2017-who-publishes-list-of-bacteria-for-which-new-antibiotics-are-urgently-needed

- https://www.who.int/publications/i/item/2021-aware-classification