Antibiotic resistance: From a broader perspective

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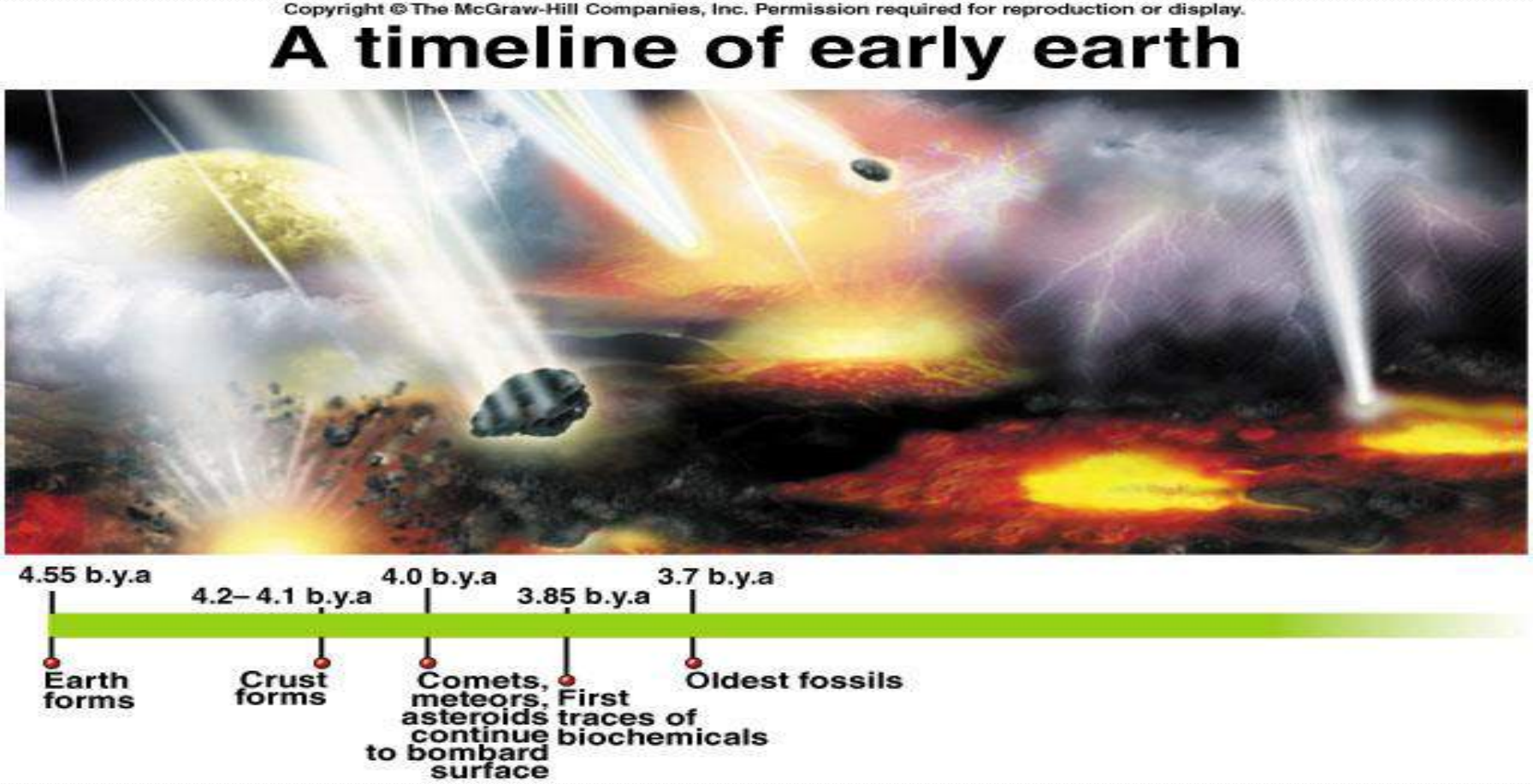
Ancestors of bacteria were the first life on Earth

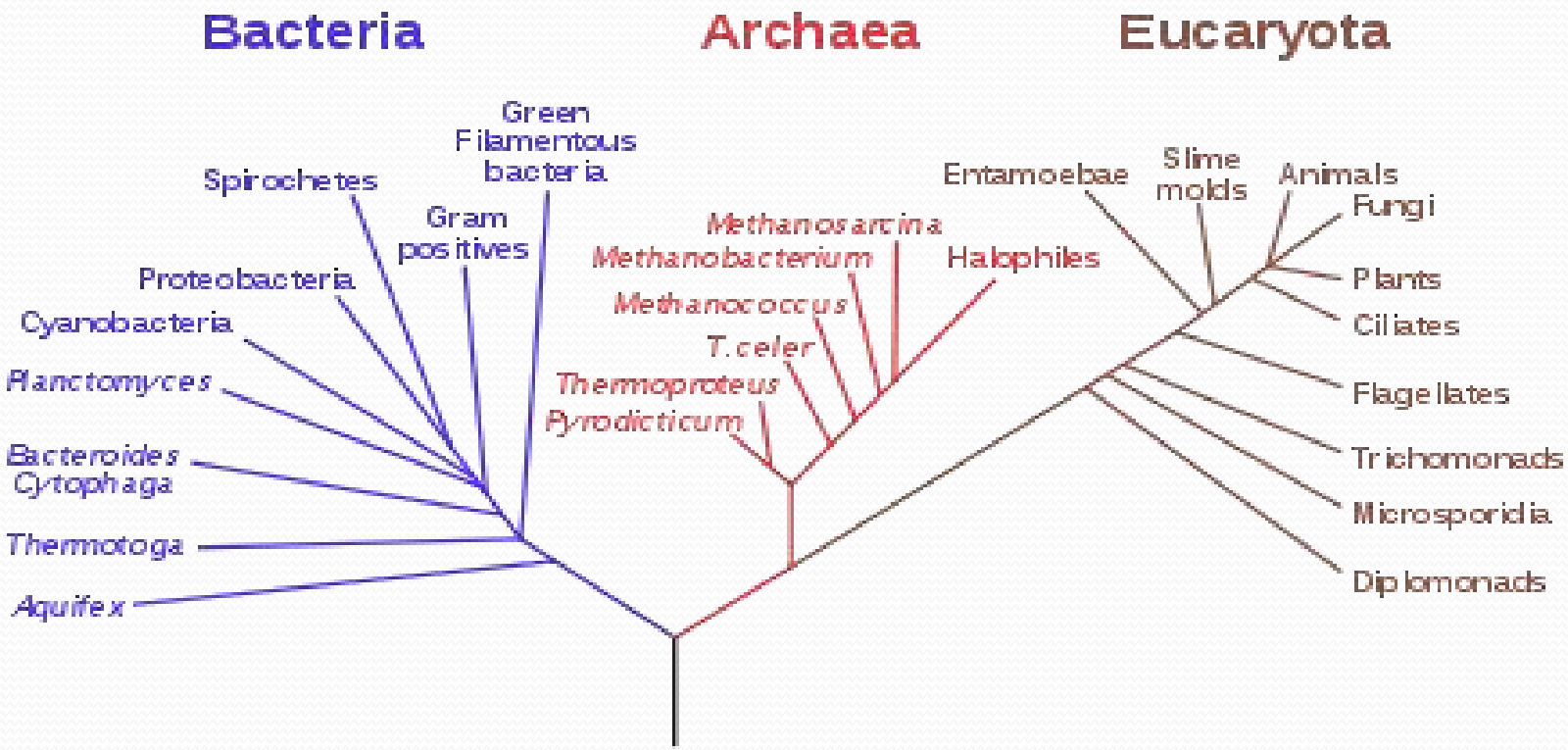


- The ancestors of modern bacteria were unicellular microorganisms that were the first forms of life to appear on Earth, about 4 billion years ago.

- For about 3 billion years, most organisms were microscopic, and bacteria and archaea were the dominant forms of life

Stromatolites, with two slices cut out by researchers.

Phylogenetic Tree of Life



First Observation of Bacteria

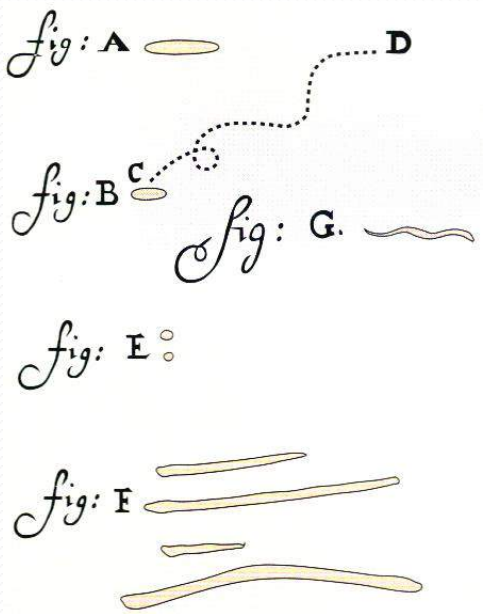
•Microorganisms were first observed about 1675 by the Dutchman Antonie van Leeuwenhoek'

- van Leeuwenhoek: 1632-1723
  - first man to view live microorganisms using a single lens microscope

Animalcules

- He described many animalcules, including the three major morphologic forms of bacteria (rod, sphere and spirals)

- Van Leeuwenhoek's "animalcules"
  - this is what he called them, based on how they moved
- drawings are representations of bacteria and protozoa



Van Leeuwenhoek drawings of animalcules: found in rainwater soaked in peppercorns and material scraped from teeth

Pioneers of antibiotic

Paul Ehrlich (1854 - 1915)

Paul Ehrlich 1854-1915

- German Physician On
- Koch's Research Team (Helped to make Koch's dye)
- Invented Chemotherapy
- Won Nobel Prize
- Made First Magic Bullet (Which was Injected) -
- Called Salvarsan 606

Gerhard Domagk

Alexander Fleming.

Cell wall of Gram positive and Gram negative bacteria

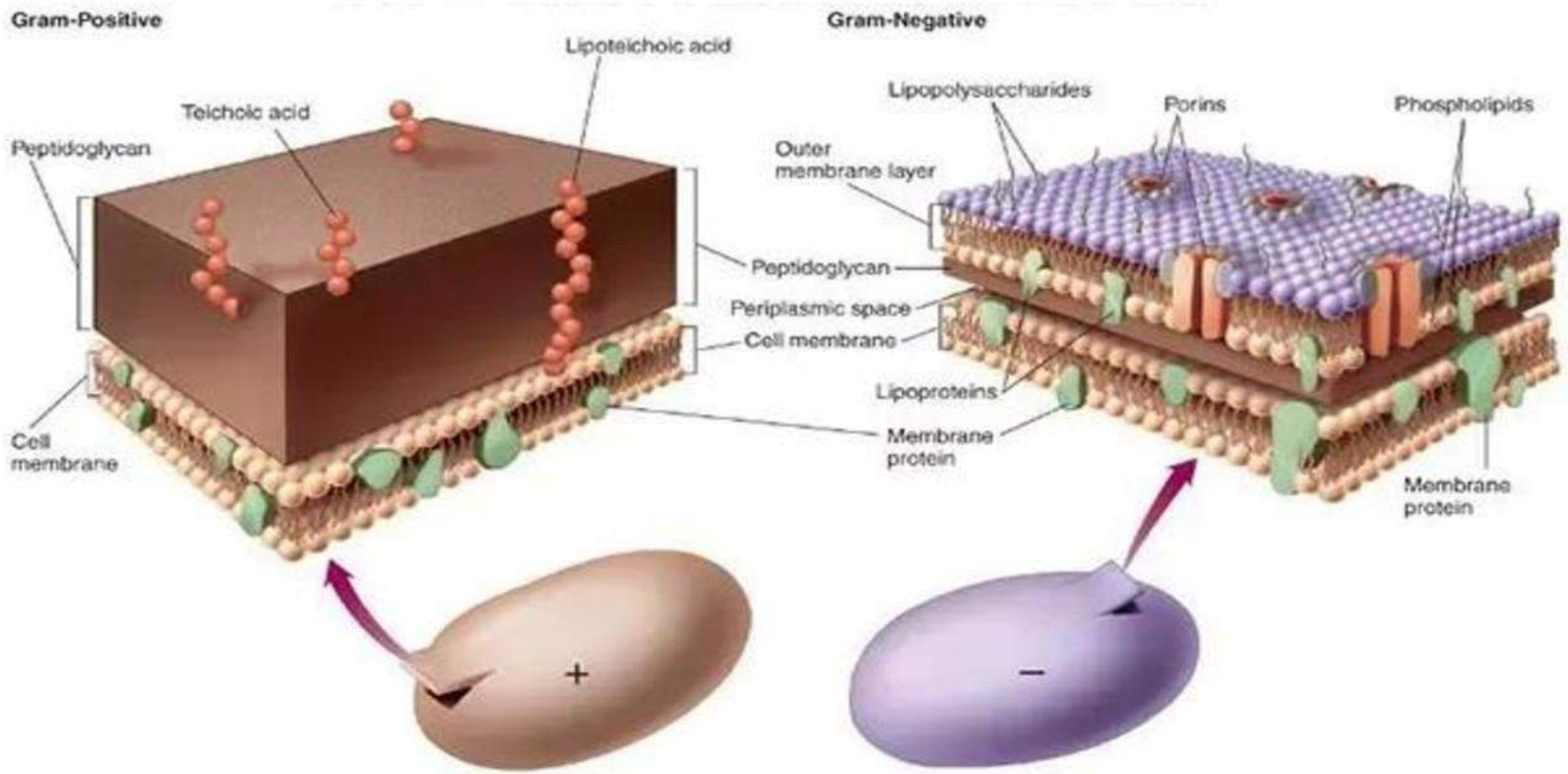
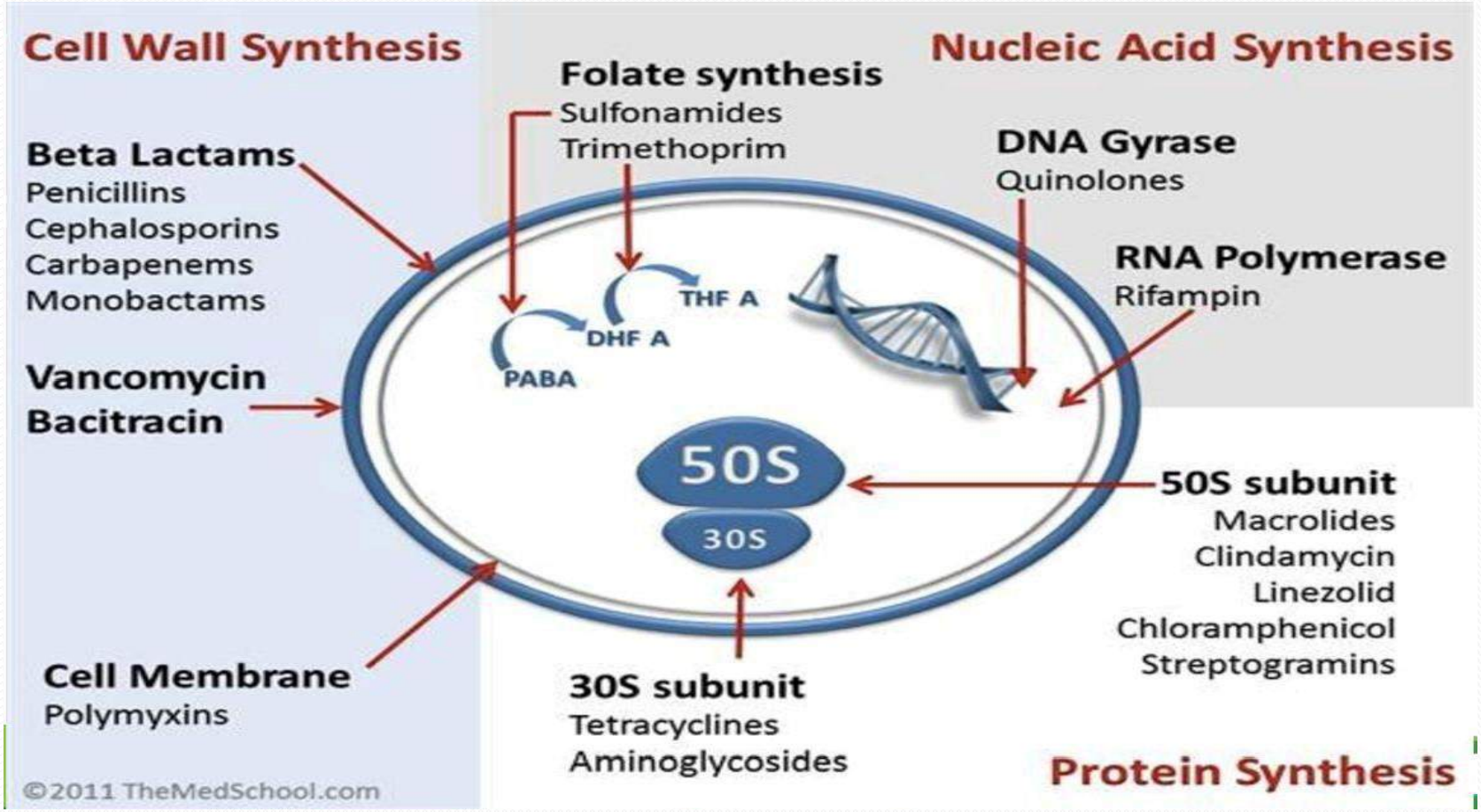


Table 1 Major antibiotic families and their mechanisms of action

Mechanism of action	Antibiotic families
Inhibition of cell wall synthesis	Penicillins; cephalosporins; carbapenems; daptomycin; monobactams; glycopeptides
Inhibition of protein synthesis	Tetracyclines; aminoglycosides; oxazolidonones; streptogramins; ketolides; macrolides; lincosamides
Inhibition of DNA synthesis	Fluoroquinolones
Competitive inhibition of folic acid synthesis	Sulfonamides; trimethoprim
Inhibition of RNA synthesis	Rifampin
Other	Metronidazole

Different methods of actions of antibiotics on bacterial cell



Resistance in microbes is a natural phenomenon

- Resistance is unresponsiveness to antimicrobial agents in standard doses
- A natural biological unstoppable phenomenon
- Resistance is generally slow to reverse or irreversible
- All antimicrobial agents have the potential to select drug-resistant subpopulations of microorganisms

What is Antimicrobial Resistance (AMR)?

Medicines for treating infections lose effect because the microbes change;

1. mutate
2. acquire genetic information from other microbes to develop resistance

Types of AMR

1. **Antibacterial resistance**	(e.g. to antibiotics and other antibacterial drugs)
2. **Antiviral resistance**	(e.g. to anti-HIV medicines)
3. **Antiparasitic resistance**	(e.g. to anti-malaria medicines)
4. **Antifungal resistance**	(e.g. to medicines used to treat *Candidiasis*)

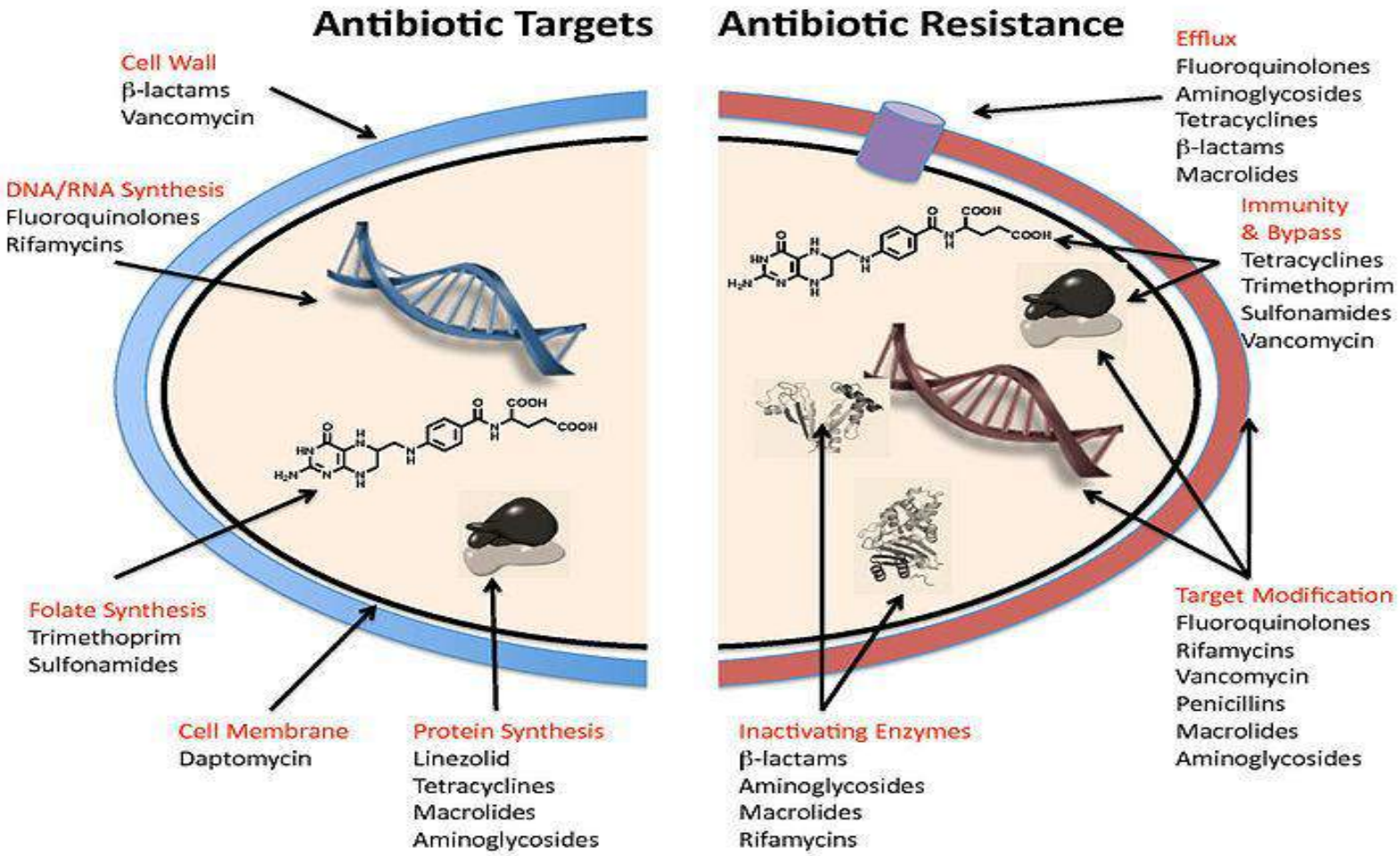
AMR is a natural phenomenon accelerated by use of antimicrobial medicines. Resistant strains survive and aggregate.

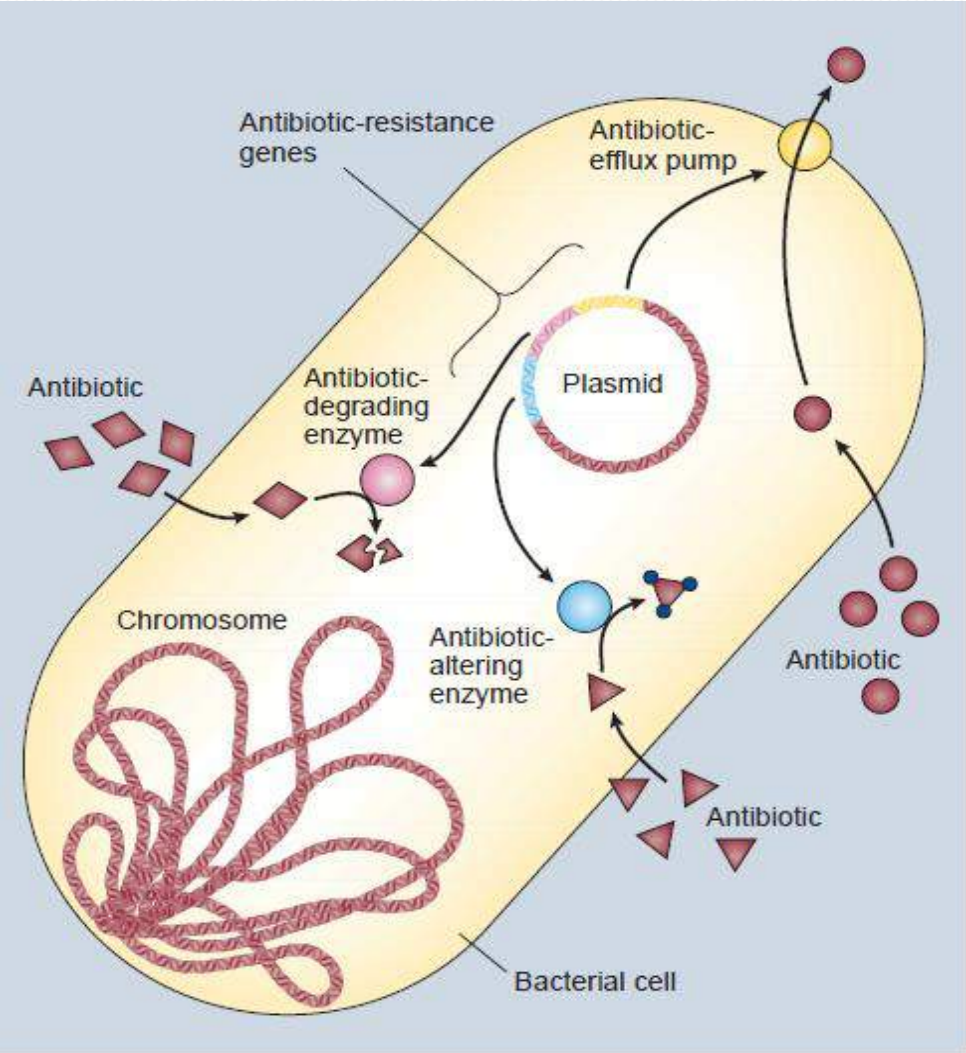
Mechanisms of drug resistance

Mechanism	Important example	Drugs commonly affected
Inactivate drug	Cleavage by beta lactamase	Beta lactum drug
Modify drug target in bacteria	1. Mutation of PBP 2. Mutation in protein in 30S ribosomal subunit 3. Replace alanine with lactate in peptidoglycan 4. Mutation in DNA gyrase 5. Mutation in RNA polymerase 6. Mutation in catalase-peroxidase	Penicillin Aminoglycosides Vancomycin Quinolones Rifampin Isoniazid

Mechanisms of drug resistance (2)

Mechanism	Important example	Drugs commonly affected
Reduce permeability of drug	Mutation in porin proteins	Penicillins, aminoglycosides and others
Export of drug from bacteria	Multidrug-resistance pump	Tetracycline, sulfonamides, quinolones


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



Biological mechanisms of resistance

Biological mechanisms of resistance

- The same kind of drug resistance mechanism can be specified by many different genes:
  - the β -lactamases now number in the hundred
  - more than 20 different resistance determinants mediate an efflux of tetracyclines.

- More than one type of mechanism may provide resistance to the same antibiotics:
  - Tetracycline resistance can be effected by either efflux or ribosome protection.
  - Most fluoroquinolone resistance stems from chromosomal mutations in the gyrase target or from drug efflux,
  - A plasmid-mediated resistance to fluoroquinolones has been recently described.

Causes of antibiotic resistance

- *The level of antibiotic-resistant infections strongly correlates with the level of antibiotic consumption*
- Irrational use of antibiotics is the greatest driver of resistance

  -50% of antibiotics are prescribed inappropriately
  - 50% of patients have poor compliance
  -50% of populations do not have access to essential antibiotics

- Domesticated animals also get infected and require antibiotic therapy.
- Antibiotics are also used for the growth promotional and prophylactic purposes in food animals.

Genetic basis of resistance

Chromosome mediated resistance:

- By mutation of gene that codes for either the target of the drug or the transport system in the membrane that controls the uptake of the drug.

- The frequency of spontaneous mutation usually ranges from 10^{-7}-10^{-9} which is much lower than the frequency of acquisition of resistance plasmid.

Genetic basis of resistance

Plasmid and transposon mediated resistance:

- Genes can be spread from one bacterium to another through various mechanisms such as plasmids, bacteriophages, naked DNA or transposons

- Chromosomal genes can be transferred: They are acquired by one bacterium through the uptake of naked DNA released from another microorganism.

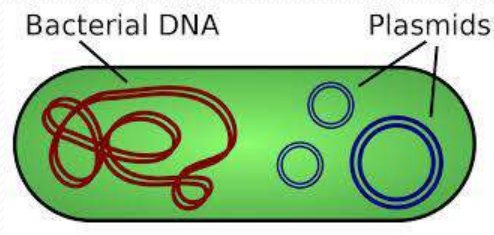
  This transfer process, called transformation.

Mobile genetic elements

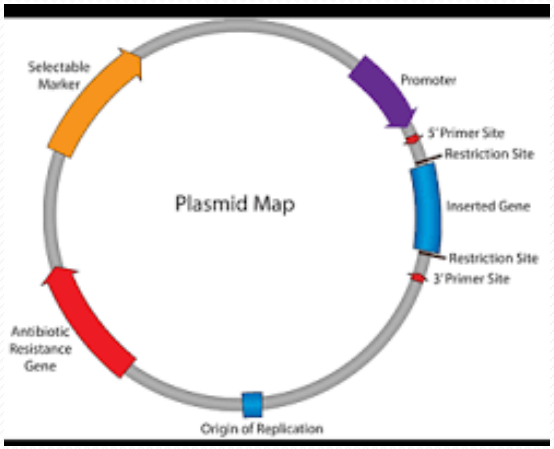
- Mobile genetic elements (MGEs) are a type of genetic material that can move around within a genome, or that can be transferred from one species or replicon to another.
- MGEs are found in all organisms.
- In humans, approximately 50% of the genome is thought to be MGEs.
- MGEs play a distinct role in evolution.
- One of the examples of MGEs in evolutionary context is that virulence factors and antibiotic resistance genes of MGEs can be transported to share them with neighboring bacteria.

Types of mobile genetic elements

Plasmid: Self-replicating, usually circular, double stranded, extra-chromosomal genetic elements that provide accessory genes, usually for non-essential functions, to their hosts.



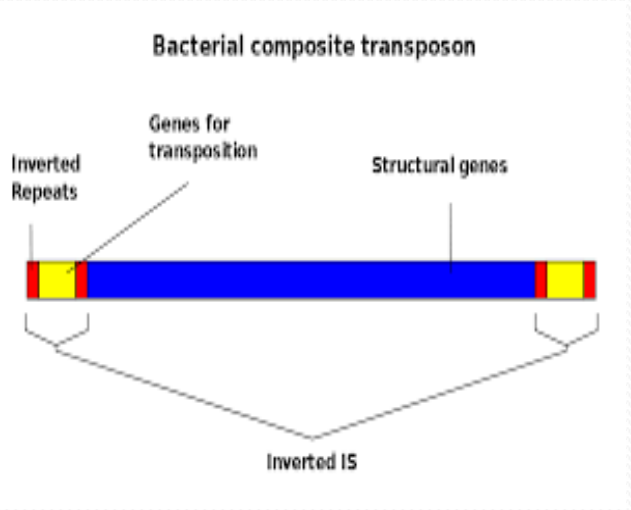
-Plasmids are not integrated into the recipient's chromosome and can continue to transfer to new hosts.



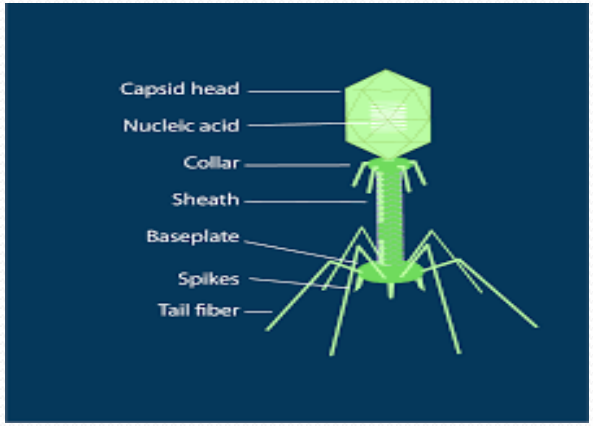
-This is a mechanism of horizontal gene transfer that allows bacteria to share virulence factors and antibiotic resistance genes.

Types of mobile genetic elements

- **Transposons:** - They are major factors of genetic reorganization and can transfer genes to other locations on the chromosomes, onto plasmids, or into phage DNA, to be transferred to a new cell



- **Bacteriophage:** It is a virus that infects and replicates within bacteria and archae.



CONSEQUENCES OF OVERUSE OR MISUSE OF ANTIBIOTICS

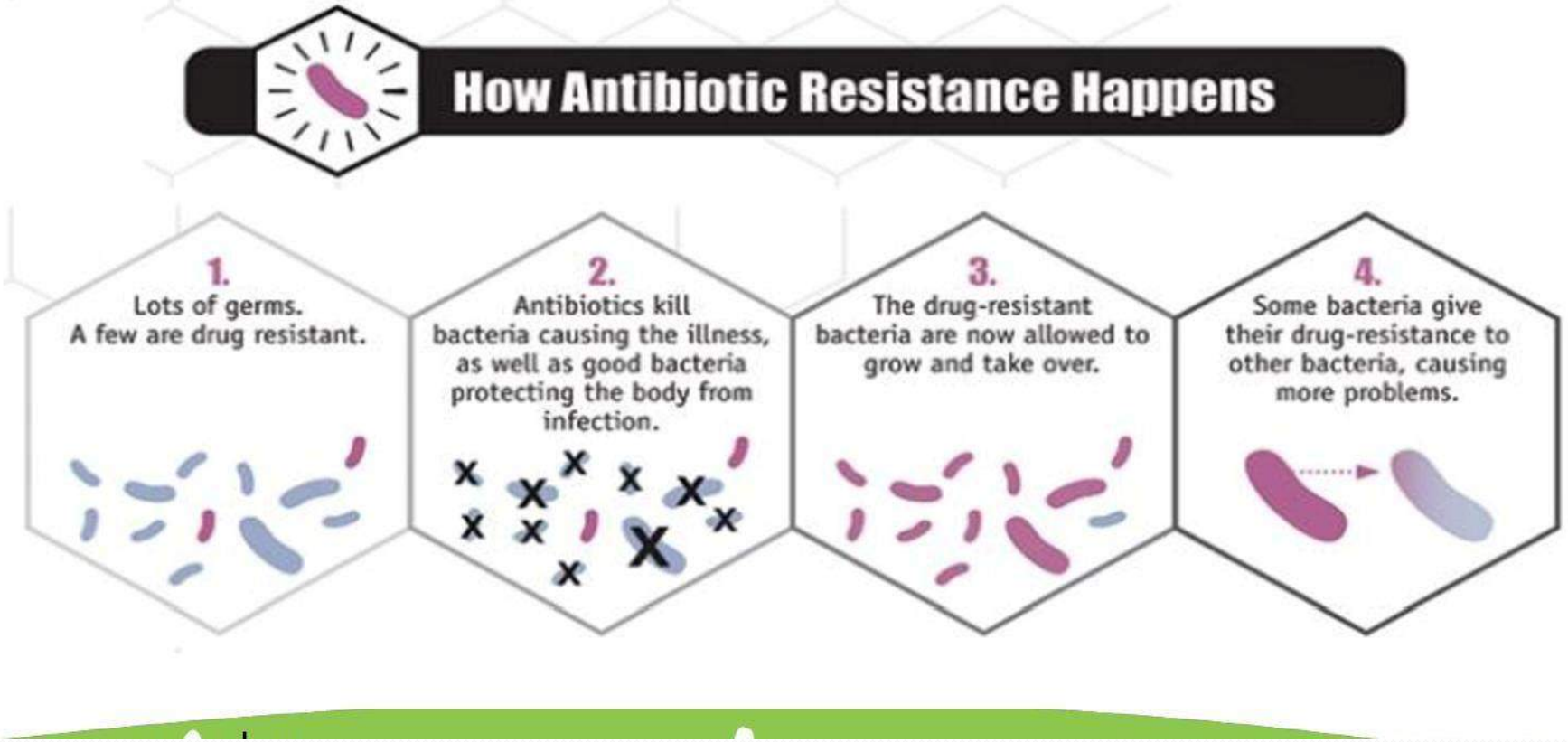


TABLE 1. Types of resistance observed in bacteria

	Intrinsic resistance	Acquired resistance
Definition	• Natural traits • Species or genus specific	• A strain that develops resistance to an antimicrobial to which it was previously susceptible • Present only in certain strains of a species or genus
Mechanisms of resistance acquisition	• Inherent structural or functional characteristics of the bacteria that allow it to tolerate or be insensitive to an antimicrobial substance or class	Vertical transmission • Spontaneous gene mutation • Induced gene mutation Horizontal gene mutation • Bacterial transformation • Bacterial transduction • Bacterial conjugation

Source: Boerlin and White, 2013

Some bacteria have intrinsic resistance

- The intrinsic resistance of a bacterial species to a particular antibiotic is the ability to resist the action of that antibiotic as a result of inherent structural or functional characteristics.
- For example, the glycopeptide antibiotic vancomycin inhibits peptidoglycan crosslinking by binding to target d-Ala-d-Ala peptides, but in Gram-negative organisms, it cannot cross the outer membrane and access these peptides in the periplasm
- *Aeromonas* has intrinsic beta-lactamases which makes them resistant to ampicillin.

Acquired antimicrobial resistance

- When microorganisms once sensitive to an antimicrobial agent become resistance to that particular antibiotic, the resistance is acquired.
- The acquired resistance could be due to genetic changes such as mutations or acquisition of genes contributing to resistance through horizontal gene transfer.

How Antibiotic Resistance Moves Directly Germ to Germ

Any antibiotic use can lead to antibiotic resistance. Antibiotics kill germs like bacteria and fungi, but the resistant survivors remain.

Resistance traits can be inherited generation to generation. They can also pass directly from germ to germ by way of **mobile genetic elements**.

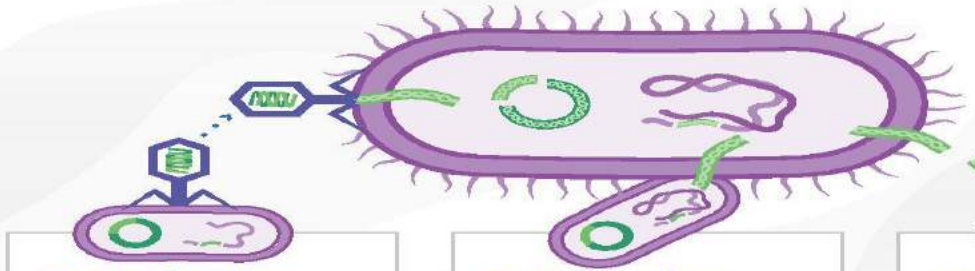
Mobile Genetic Elements

Plasmids
Circles of DNA that can move between cells.

Transposons
Small pieces of DNA that can go into and change the overall DNA of a cell. These can move from chromosomes (which carry all the genes essential for germ survival) to plasmids and back.

Phages
Viruses that attack germs and can carry DNA from germ to germ.

How Mobile Genetic Elements Work

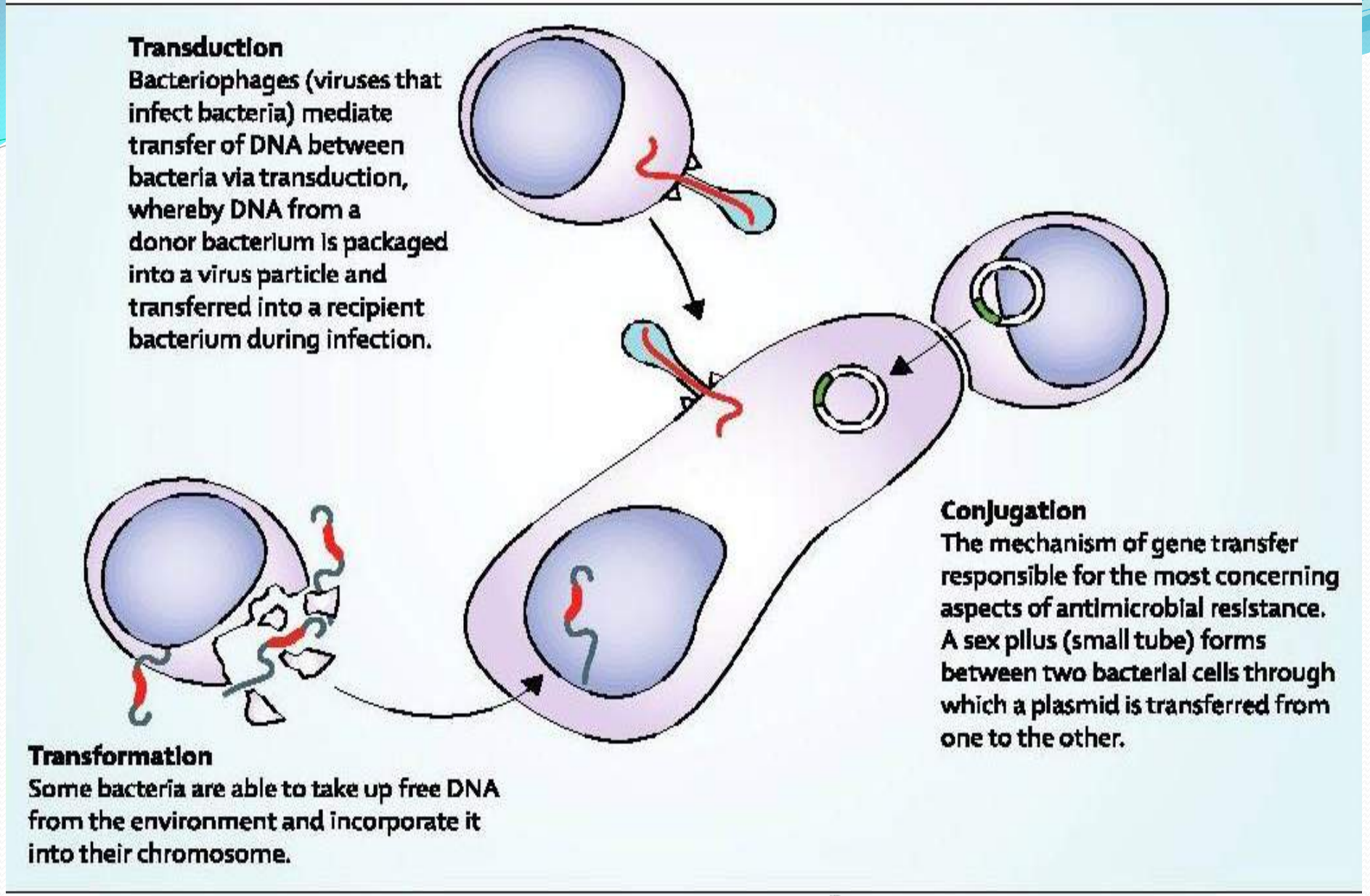


Transduction
Resistance genes can be transferred from one germ to another via phages.

Conjugation
Resistance genes can be transferred between germs when they connect.

Transformation
Resistance genes released from nearby live or dead germs can be picked up directly by another germ.

U.S. Department of Health and Human Services
Centers for Disease Control and Prevention


Transduction
Bacteriophages (viruses that infect bacteria) mediate transfer of DNA between bacteria via transduction, whereby DNA from a donor bacterium is packaged into a virus particle and transferred into a recipient bacterium during infection.
Conjugation
The mechanism of gene transfer responsible for the most concerning aspects of antimicrobial resistance. A sex pilus (small tube) forms between two bacterial cells through which a plasmid is transferred from one to the other.
Transformation
Some bacteria are able to take up free DNA from the environment and incorporate it into their chromosome.

Resistance genes have other functions in the cell

- ampC beta-lactamase is involved in maintaining normal morphology in *Escherichia coli*.
- Efflux pumps are involved in efflux of several compounds
- bla_{oxy} beta-lactamase has metabolic function in *Klebsiella oxytoca*

Resistome

- The use of antimicrobials in clinical practice is a recent development in history compared to the emergence of bacterial organisms on our planet.
- Therefore, development of antibiotic resistance should be viewed as a "normal" adaptive response and a clear manifestation of Darwinian's principles of evolution.
- Although no broad baseline data collection on antibiotic resistance genes has been performed at the onset of antibiotic use, recent studies suggest that the most likely source of these genes is the environmental antibiotic **resistome**

Resistome

- Bacteria do not respect the boundaries of ecological compartments
- There is always a continuous flow by genetic information between different ecological compartments.
- Once the potential antibiotic resistance genes enter, even in small numbers or low frequencies, into the commensal/pathogenic human/animal microbiota, the antibiotic selection immediately leads to the amplification and dissemination of these genes.
- *Indeed, there is some evidence on the environmental origin of some clinically relevant resistance genes*

Resistome

The **resistome** is a proposed expression by Gerard D.Wright for the collection of all the antibiotic resistance genes and their precursors in both pathogenic and non-pathogenic bacteria. They are:

1. Found on **pathogenic bacteria**. These are the fewest but also the most problematic

2. Found on **antibiotic producers**. The microorganisms such as soil-dwelling bacteria and fungi that naturally produce antibiotics have their own protection mechanisms to avoid the adverse effects of the antibiotics on themselves.

The genes which code for these resistances are a strong source for the pathogenic bacteria.

Resistome

3. Cryptic resistance genes. These genes are embedded in the bacterial chromosome but do not obviously confer resistance, because their level of expression is usually low or they are not expressed.

4. Precursor genes. These genes do not confer antibiotic resistance.

However they encode proteins that confer to some kind of basal level activity against the antibiotic molecule or have affinity to the molecule.

In both cases this interaction may evolve to a full resistance gene given the appropriate selection pressure.

Human GI microbiome diversity and the “resistome”

- A recent study of a previously un contacted isolated hunter-gatherer community confirmed that, despite the lack of contact with clinical antibiotics and pharmaceuticals, the microbiomes of isolated communities harbor resistance genes.
- This suggests that exposure to clinical antibiotics is not necessary to spread resistance and the gut microbiome can be shaped by **natural antibiotics** that may result in cross-protection of gut organisms from clinical antibiotics.
- Likewise, antimicrobial agents utilized in medicine can be applied to combat pathogenic bacteria but **this introduction of antibiotics to the gut can also result in a reduction of susceptible commensal organisms**
- “Hitchhiker”organisms that enter the gut ecosystem from ingestion of water or food can include pathogens and other organisms that can hold ARGs, allowing opportunities for these genes to be passed on to commensal members of the intestine

Development of AMR and spread of antibiotics

- In the absence of plasmids and transposons (which generally mediate high-level resistance), a step-wise progression from low-level to high-level resistance occurs in bacteria through sequential mutations in chromosomes.
- Resistant bacteria accumulate multiple resistance determinants.
- The long-term use of a single antibiotic (that is, for more than 10 days) will select for bacteria that are resistant not only to that antibiotic, but to several others.
- This phenomenon was found to occur after the prolonged use of tetracycline for urinary tract infections and for acne.

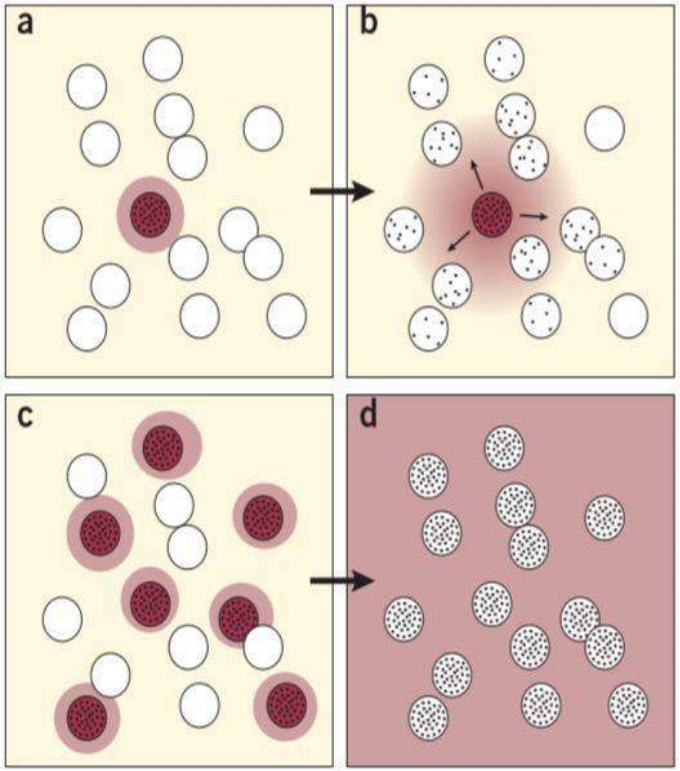
Development of AMR and spread of antibiotics

- Loss of resistance is slow. Resistant bacteria may rapidly appear in the host or environment after antibiotic use, but they are slow to be lost, even in the absence of the selecting antibiotic.
- A significant countrywide reversal of macrolide resistance in *S. pyogenes* resulted from a Finnish nationwide campaign to reduce macrolide usage.
- In 2 years, resistance declined from about 20% to less than 10% .
- Replacement by susceptible flora represents a chief contribution to a decrease in resistant strains.
- *The fastest way to eliminate resistant strains is to outnumber them with susceptible strains.*

Development of AMR and spread of antibiotics

- Ecologically speaking, it is the density of antibiotic usage that enhances resistance selection and its effects.
- The ‘selection density’ involves the total amount of antibiotic being applied to a geographically defined number of individuals in a setting, whether it is the home, daycare center, hospital or farm
- Each individual becomes a ‘factory’ of resistant bacteria that enter the environment.
- The disparity between resistance rates in the local community and those in city hospitals reflects differential ecological effects of antibiotic use.

Post-therapeutic effects of antibiotic dispersion:



(a) While on antimicrobial therapy, the individual (e.g. person or animal) is a focal point for a high concentration of both antibiotic (red shading) and resistant bacteria (black dots) that are selected and generated from its use.

(b) Over time, resistant bacteria spread to local contacts and antibiotic enters the environment through waste and water disposal (for example, from animals) or sewage (from people). If several individuals are treated

(c) A higher density of antimicrobial and resistant organisms is established in the same environment

(d) The selective process is continuous, occurring both during and after therapy

Development of AMR and spread of antibiotics

- For example, antibiotic treatment for acne was found to produce an MDR skin flora not only in the individual with acne, but also in other members of the household.
- High numbers of MDR bacteria were found in the intestinal flora of ambulatory individuals in the Boston area, even though none had recently taken an antibiotic.
- Antimicrobials in waste waters are being reported with increasing frequency and are potentially important contributors to the environmental selection of antibiotic-resistant organisms

Commensal bacteria as reservoir

- *Commensal organisms are common reservoirs of antibiotic resistance plasmids, transposons and genes.*
- *E. coli* and the enterococci of the gut serve as reservoirs from which several antibiotic resistance genes can spread
- *Staphylococcus epidermidis* serves as a reservoir for resistance genes and plasmids for the more pathogenic *S. aureus*.
- Vancomycin resistance determinants found initially among enterococci appeared in other commensal bacteria before emerging in *S. aureus*.

Introduction

- Emergence of resistance among the most important bacterial pathogens is recognized as a major public health threat affecting humans worldwide.
- Multidrug-resistant organisms have emerged not only in the hospital environment but are now often identified in community settings, suggesting that reservoirs of antibiotic-resistant bacteria are present outside the hospital.
- The bacterial response to the antibiotic "attack" is the prime example of bacterial adaptation.
- Immense genetic plasticity of bacterial pathogens trigger specific responses that result in resistance to virtually all antibiotics currently available in clinical practice.

Introduction

- World Health Organization has named antibiotic resistance as one of the three most important public health threats of the 21st century

- This situation is worsened by a paucity of a robust antibiotic pipeline, resulting in the emergence of infections that are almost untreatable and leaving clinicians with no reliable alternatives to treat infected patients.

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Antimicrobial resistance is ancient, natural and is found in environments with no exposure to antibiotics

- Viable multidrug-resistant bacteria have been cultured from the Lechuguilla Cave in New Mexico, totally isolated for >4 million years (Bhullar et al., 2012).
- Antibiotic resistant marine bacteria have been found as far as 522KM offshore and in deep sea at depths of 8200m (Aminov, 2011).
- Evolution of antibiotic resistance genes predates evolution of Actinomycetes.
- Some of the antibiotic resistance genes have not evolved to protect against antibiotics but have other metabolic functions.

Resistance genes found in environmental bacteria without exposure to antibiotics

- *qnr* gene conferring resistance to quinolones are found in marine bacteria like *Shewanella algae* and *Vibrio* spp.
- CTX-M beta-lactamase is present in environmental bacteria like *Kluyvera*

Which came first, the antibiotic or resistance?

- Penicillin was discovered by Alexander Fleming in 1928,
- In 1940 several years before the introduction of penicillin as a therapeutic, a bacterial penicillinase was identified by two members of the penicillin discovery team.
- Antibiotics and antibiotic resistance determinants are a natural phenomenon and have been present in the environment long before humans discovered and begun to use antibiotics.
- Microorganisms produce antibiotics to gain a growth advantage and to defend against competing organisms
- Antibiotics also act as messenger molecules in microbial communities, for instance, in quorum sensing.

MIC and MSC

- In clinical settings, it is most common to refer to minimum inhibitory concentrations (MIC) for antibiotics, above which bacterial growth inhibition is observed.
- The effects that antibiotics mediate in an ecosystem, however, occur at much lower concentrations.
- It has been shown that even *very low concentrations of antibiotics are sufficient to provide a selective advantage for resistant over non-resistant microorganisms.*
- It is called minimum selective concentrations (MSC) to characterize the effect of antibiotics in the environment

Natural antibiotic and its resistance

- The natural environment harbors a diverse reservoir of resistance determinants, including resistance genes and the mobile genetic elements that operate as vectors for them.
- Commonly, gene clusters that encode the proteins required to synthesize an antibiotic also code for self-protection mechanisms
- Various resistance genes have a long phylogenic history dating back millions of years, but resistant genotypes also arise from scratch by mutation

Superbugs and Super resistance

- Many of the bacterial pathogens associated with epidemics of human disease have evolved into multidrug-resistant (MDR) forms subsequent to antibiotic use.
- For example, MDR *M. tuberculosis* is a major pathogen found in both developing and industrialized nations and became the 20th-century version of an old pathogen.
- Microbes resistant to multiple antimicrobials are called multidrug resistant (MDR).
- Those considered extensively drug resistant (XDR) or totally drug-resistant (TDR) are sometimes called "superbugs".

MDR

The isolate is non-susceptible to at least 1 agent in ≥3 antimicrobial categories

XDR

The isolate is non-susceptible to at least 1 agent in all but 2 or fewer antimicrobial categories

PDR

Non-susceptibility to all agents in all antimicrobial categories for each bacterium

Superbugs And Super resistance

- The term “superbugs” refers to microbes with enhanced morbidity and mortality due to multiple mutations endowing high levels of resistance to the antibiotic classes specifically recommended for their treatment;
- The therapeutic options for these microbes are reduced, and periods of hospital care are extended and more costly.
- In some cases, super resistant strains have also acquired increased virulence and enhanced transmissibility.
- *Realistically, antibiotic resistance can be considered a virulence factor.*

Superbugs And Super resistance

- Conventionally, the struggle against antibiotic resistance development has mainly taken place in clinical, community, and in more recent years also agricultural.
- *Over the past years, the role of the environment as an important source and dissemination route of resistance has been increasingly recognized.*
- Our understanding of its contribution is still limited

The Origins Of Resistance Gene

- Antibiotic resistance genes (ARGs) should be considered a biological contaminant of emerging concern(CEC).
- *Natural antibiotics are thought to have first appeared as many as 2 billion years ago, and antibiotic resistance is just as ancient and predates the use of antibiotics in agriculture and medicine*
- Antibiotic resistance only became part of the public consciousness when clinical resistance emerged
- ARGs are not a new occurrence and that the abundance of some of these genes has changed over time with the widespread use of antibiotics.

The Origins Of Resistance Gene

- *Novel antibiotic resistance factors could potentially emerge anywhere, at any time.*
- The astounding number of bacterial cells on Earth, estimated to around 10^{30}—a thousand billion billion billions , provide an immense genetic variability, and opportunities for mutations, rearrangements and horizontal gene transfer.
- Thus, new resistance factors likely appear regularly, although we never detect the vast majority of these events.

The Origins Of Resistance Gene

- Since the beginning of the antibiotic era in the first half of the 20th century, antibiotics and antibiotic resistance genes have been introduced to or have spread to almost every ecosystem on earth.

- Another important aspect is the co-selection of antibiotic resistance by the presence of heavy metals or biocides that are also anthropogenically introduced into the environment

- But there are nevertheless several reasons to why pathogens are not flooded by novel resistance genes.

- For a start, resistance factors are generally associated with some *fitness cost*.

Fitness cost

- This cost may be particularly large for genes providing novel resistance functions for a bacterium, as their expression may not be sufficiently fine-tuned and their products may interfere with other cellular functions.
- Thus, novel resistance genes will be selected against unless there is a relatively strong selection pressure to maintain them.
- Furthermore, even if such a resistance factor would have a low or negligible fitness cost, it would still be rare, and may therefore not become permanently established in the bacterial population unless there is a positive selection pressure for it.
- This selection pressure may be weak, but unless it is present the only way by which a novel resistance factor would be retained is through genetic drift

Fitness cost

- For example consider an antibiotic which targets an important biological pathway. Mutations that confer antibiotic resistance often involve modification of the target enzyme to prevent antibiotic binding.

- These mutations often make enzyme suboptimal compared to evolutionary optimized "wild-type" version. This can reduce fitness, manifesting as decreased virulence, transmission, and growth rate.

- However, despite being less fit under normal growth conditions, this mutant can survive under conditions of antibiotic treatment. So this a trade off also known as fitness cost.

- The cost to the ‘fitness’ of an organism is it’s ability to replicate and survive in a competitive environment.

Fitness cost

- *For instance if antibiotic resistance could be acquired by bacteria without any "fitness cost" all the human bacteria (as well as all the environmental ones) would be pan-resistant already.*

- Bacteria are masters of survival. They are also hyper-efficient, and need to carefully budget their energy quota not only on defense, but also on attack and redeployment, not to mention communication (fitness cost).

- If the bacterial population does not need to be resistant in a particular environment, it will not waste its precious energy (and time!) on resistance genes for a “Just in case” scenario.

- You can produce sophisticated and comprehensive antimicrobial resistance surveillance data.
- You can adhere to the best infection control policies in the country.

- You can have a "search and destroy" policy for multi-resistant organisms.

and

- You can even develop and bring out a new antibiotic every couple of years….

but……

Unless you control antibiotic consumption (usage), you will always be fighting an uphill battle.

Horizontal Transfer of Resistance Factors

- Horizontal gene transfer is central for the spread of novel (and known) resistance genes, as it allows resistance to expand beyond specific clones.
- This way, gene transfer makes resistance genes available to a much larger part of the bacterial community in a particular environment, often beyond species boundaries.
- As for the mobilization of resistance factors, transfer of genes between bacteria can in theory occur anywhere.
- However, for resistance genes to be horizontally transferred from environmental to pathogenic bacteria they need to, at least temporarily, share the *same habitat*.
- Furthermore, horizontal gene transfer is much more likely to occur between *phylogenetically closely related bacteria*.

Horizontal Transfer of Resistance Factors

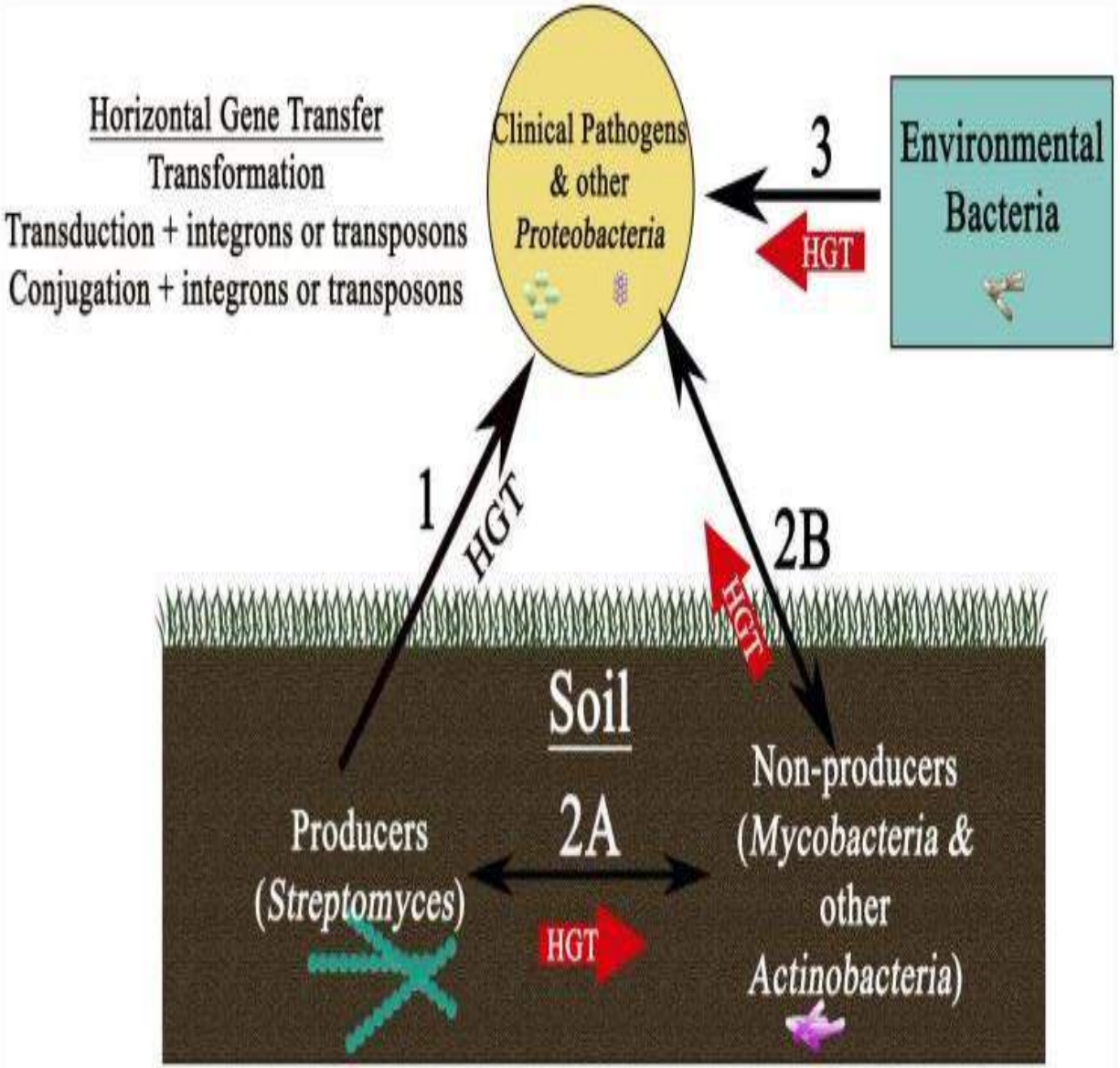
- *Finally, transfer of genetic material between bacterial cells is induced by stressors such as antibiotics and potentially also metals and biocides.*
- Subsequently, antibiotic selection also contributes to establishment of transferred resistance genes in their new host.
- Thus, resistance transfer to pathogens could be expected to be relatively frequent between human-associated bacteria, particularly during treatment with antibiotics.
- In contrast, transfer of resistance genes to pathogens from environmental bacteria, which occupy other habitats and are often less phylogenetically related, would likely be less common
- *Although environmental stressors may induce horizontal gene transfer to and from (opportunistic) human pathogens in environmental settings*

Dissemination Of Resistant Bacteria

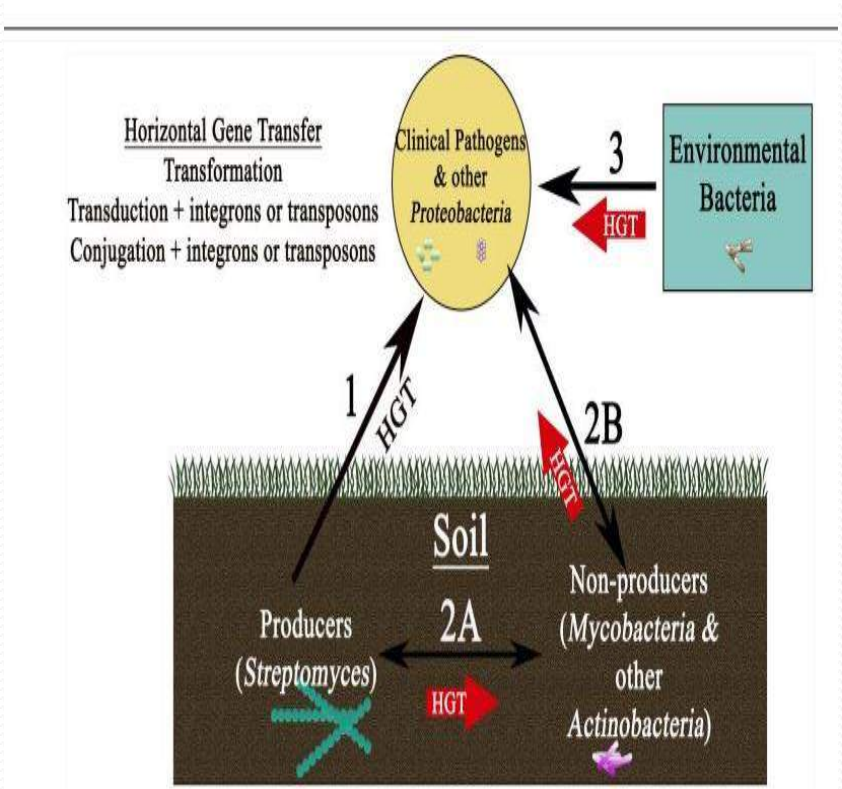
- The main route of exposure for humans to resistant pathogens is from other people, either in clinics or through the community setting.
- Typical dispersal routes here are through body contact or indirect contact transmission, aerosols, and food prepared by persons carrying the pathogen.
- These are also the typical transmission routes for infectious bacteria in general
- *Proper hygiene routines and sanitation constitute the principal dispersal barrier for resistant pathogens,*

Dissemination Of Resistant Bacteria

- Apart from transmission between humans, environmental dissemination routes for resistant bacteria have also been pointed out as potentially important for the spread of antibiotic resistance.
- Environments facilitating dissemination of resistant bacteria also enable spread of non-resistant human pathogens, and generally also opportunistic pathogens
- Thus, sewage, wastewater treatment plants, water bodies and travel, but also air-borne aerosols, dust, and food colonized by bacteria, are important vectors enabling bacterial transmission between hosts through the environment

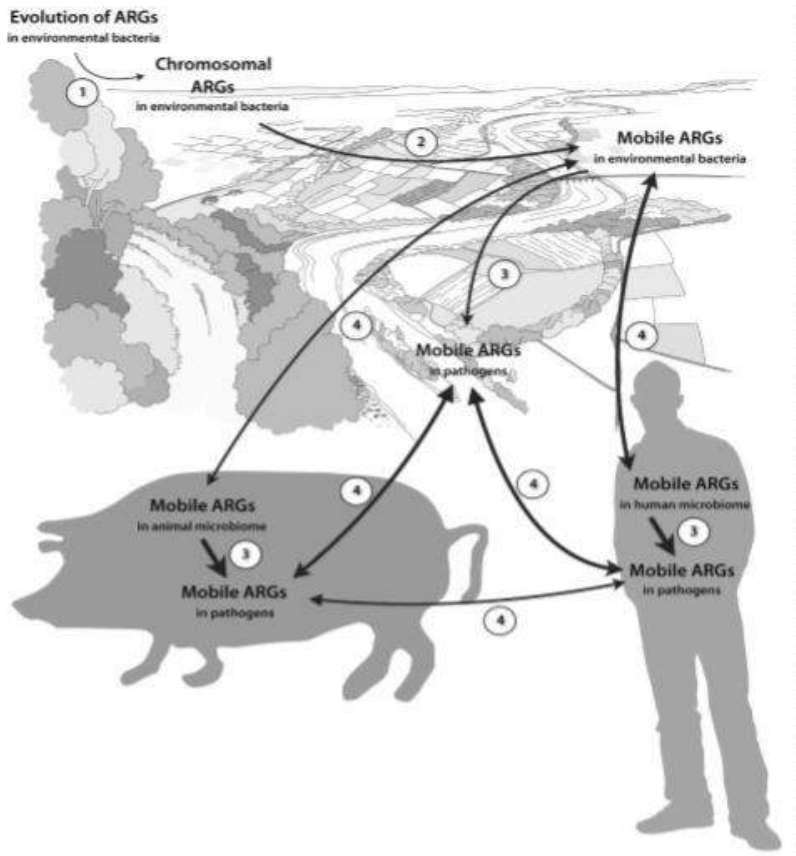

Horizontal Gene Transfer
Transformation
Transduction + integrons or transposons
Conjugation + integrons or transposons
Clinical Pathogens & other Proteobacteria
3
HGT
Environmental Bacteria
1
HGT
2B
HGT
Soil
Producers (Streptomyces)
2A
HGT
Non-producers (Mycobacteria & other Actinobacteria)

Antibiotic resistance gene and their movement



- Schematic showing reservoirs of antibiotic resistance genes found in nature and various pathways for their movement to the clinic.
- Transfer of resistance genes to clinical isolates could occur by a variety of routes (shown by arrows), each using horizontal gene transfer mechanisms potentially involving plasmids, integrons or transposons.
- Direct transfer of resistance determinants from producers in the soil to clinical strains is possible (Route-1),
- A more likely route may first involve movement from the producer soil bacteria to non-producer soil bacteria (for example Mycobacterium species) (Route-2A),
- Followed by transfer to clinical pathogens through several carriers (Route-2B).
- Another, possibly more important route could involve direct transfer from environmental bacteria (found in bodies of water, aquaculture, livestock animals, wildlife and plants) to clinical isolates (Route-3).
- Routes 2 and 3 are shown as thick red arrows, implying greater probability of these pathways for dissemination of resistance genes to clinical strains.

Antibiotic resistance gene and their movement



The role of the environment in the recruitment of antibiotic resistance genes (ARGs) to human pathogens includes four major steps:

(1) Emergence of novel resistance factors in the environment

(2) Mobilization onto mobile genetic elements

(3) Transfer of ARGs to human pathogens

(4) Dissemination of ARGs into the human microbiome.

Dissemination Of Resistant Bacteria

- STPs generally discharge their effluent (which has repeatedly been shown to contain resistance genes) into water bodies.
- Water contaminated by STP effluents is often used for irrigation of farmland, for recreational swimming and as drinking water supply (after further treatment).
- Domestic animals often drink such surface water untreated and may subsequently spread resistant bacteria to humans.
- However, for the dissemination of resistant bacteria, untreated sewage released into water bodies poses a considerably larger risk than STP effluents,

Dissemination Of Resistant Bacteria

- The environment is increasingly being recognized for the role it might play in the global spread of clinically relevant antibiotic resistance.

- Environmental regulators monitor and control many of the pathways responsible for the release of resistance-driving chemicals into the environment (e.g., antimicrobials, metals, and biocides).

Environments associated with ARG dissemination

- The abundance and diversity of ARGs found in clinical pathogens are relatively small compared to the diversity found in the environment .
- Mobilization of ARGs in the environment represents a significant potential risk to human and animal health.
- The most likely environments to encourage the transfer of ARGs to pathogenic bacteria are those in which pathogenic and environmental bacteria can interact .
- These environments can be classified **as "genetic reactors".**
- These "genetic reactors" are further referred to either as *hotspots* for gene transfer or as *reservoirs* of ARGs.

Reservoirs and hotspot

- Hotspot: Places where gene transfer occurs at high rates such that the system or environment is conducive to the production of high numbers of AROs.
- Reservoir: Environments that act as repositories or facilitate the long-term storage of antibiotic resistance determinants.
- The GI tract and WWTPs, are environments with dual functions as reservoirs and hotspots.
- Soil and sediment act as environments that serve as reservoirs.
- Finally, *aquatic environments helps dissemination of antibiotic resistance determinants and acts as a transport vessel for ARGs to new environments*.
- Each of these environments plays a role in ARG dissemination

Animal digestive systems: point sources for ARGs

- The emergence and selection of resistance in bacteria from animals subjected to antibiotic regimens suggest that since the introduction of veterinary antibiotics, the resistance in pathogenic and faecal bacteria has increased
- For example, **Avoparcin** was once a commonly used antibiotic for growth promotion in pigs and poultry in Europe. Its use coincides with the increased prevalence of resistance to similar antibiotics in faecal enterococci.
- Avoparcin has never been used for medical applications but has been suggested to contribute to vancomycin resistance in enterococci associated with human infections
- Both vancomycin and avoparcin are glycopeptides and the ARGs for the two antibiotics share similar mechanisms for resistance

Animal digestive systems: point sources for ARGs

- *There is lack of high levels of VRE in faecal samples of healthy animals and humans in countries that have never used this antibiotic in agriculture, such as Sweden and the US*
- Haugetal.(2011)were able to demonstrate the transfer of resistance genes in a colonic fermentation model
- Antibiotic resistant organisms associated with the gut can spread to other environments in faecal matter in the form of sewage, manure and fertilizers.

Wastewater treatment plants (WWTPs)

- Link between antibiotic use and ARG dissemination is clear in human and animal systems, but in case of WWTPs
- The WWTPs create a microbial environment conducive to high degrees of gene transfer which could facilitate a high degree of resistance in bacteria isolated from WWTP effluent and the abundance of some AROs in waste water.
- Regulations and procedures for disinfection of sewage effluent are designed to ensure that the majority of pathogenic bacteria that can survive the treatment process are removed from the effluent before it is discharged into receiving waters.
- Regulations are based on the detection of indicator species, usually E. coli.
- While disinfection can be effective in removing live organisms, it may select for AROs or release ARGs from organisms that can later be taken up through HGT or enter the sediment in the receiving water body.

Wastewater treatment plants (WWTPs

- The role of antibiotics in the propagation of ARGs in WWTPs and the overall environmental impact of the low concentrations of antibiotics on microbial communities found in this environment have been disputed in numerous studies.
- One of the primary pathways for residues of pharmaceuticals(like antibiotics) to other environments (soil and water) is via discharges from WWTPs or land application of sewage sludge and animal manure.
- It is reasonable to assume that AROs and ARGs may also use this pathway to enter other environments, providing another point source for these contaminants.
- In summary, the abundance of AROs and ARGs in sewage effluent has been well-established, but the factors that contribute to the gene transfer responsible for their increased prevalence are not well-understood and are a constantly evolving area of research

Soil and sediment: reservoir and nonpoint source for ARGs

- Soil environment plays an important role in both the fate of antibiotics and antibiotic resistance development.
- The soil fungus Penicillium chrysogenum (previously known as P. notatum) was the source of penicillin, the first naturally-occurring antibiotic to be used commercially.
- The current research regarding ARGs and AROs in soil, and the unknown extent of antibiotic production, suggests that *soil environments may provide the best example of a reservoir for ARGs* .

Soil and sediment: reservoir and nonpoint source for ARGs

- This is significant to the classification of ARGs as a CEC (contaminants of emerging concern) because soil was the original environment for organisms harbouring many of these genes.
- The spread to other environments over a short period of time could be indicative of human activity influencing the spread of ARGs into other environments and posing a public health risk.
- Rather than as a mixed environment like the GI tract and WWTPs, soil acts as a reservoir for new antibiotics and resistance genes.

Where have all the antibiotics gone?

- In the year 2000, antibiotic production in the United States totalled 50 million pounds. Assuming this level of production for the past 20 years, it can be estimated that one billion pounds were made during this time.
- When we consider China, India and other countries, the quantity of antibiotics produced and used worldwide may be at least three times greater.
- The total amount of antibiotics produced since the beginning of the antibiotic era in 1950 is obviously very considerable, and may be significantly more than what is produced naturally in the biosphere.
- *Approximately 50% of this has been devoted to human use,* with the remainder applied in animal husbandry, agriculture and aquaculture, etc
- In the face of this flood of bioactive molecules, bacteria have prevailed and even flourished through a variety of mechanisms of genetic jugglery with the concomitant selection of very high levels of antibiotic-resistant organisms in the biosphere.

Where have all the antibiotics gone?

- The life cycle of pharmaceutically used antibiotics does not simply end when a patient swallows a pill or when livestock are treated.
- In most cases, the antibiotics are excreted.
- The exact amount varies depending on the route of application and the species, but various estimates of active compounds being excreted in urine or feces range from 10% to more than 90%.
- For some highly consumed antibiotic classes, such as beta-lactams, tetracyclines, (fluoro) quinolones, phenicols and trimethoprim, excretion generally exceeds 50% of the administered dose

Where have all the antibiotics gone?

- Antibiotics and their metabolites excreted by patients go through the sewage system to waste-water treatment plants (WWTPs)
- Even a three-step—mechanical, biological and chemical—treatment is not sufficient to remove all pharmaceutical residues, including antibiotics
- Although some antibiotics, especially fluorochinolones, are removed from the water phase, they accumulate in sewage sludge.

- Antibiotics can therefore either leave the WWTP in treated water that enters rivers and lakes, or they become part of the sewage sludge and are introduced into the environment when the sludge is used as fertilizer or as filling material

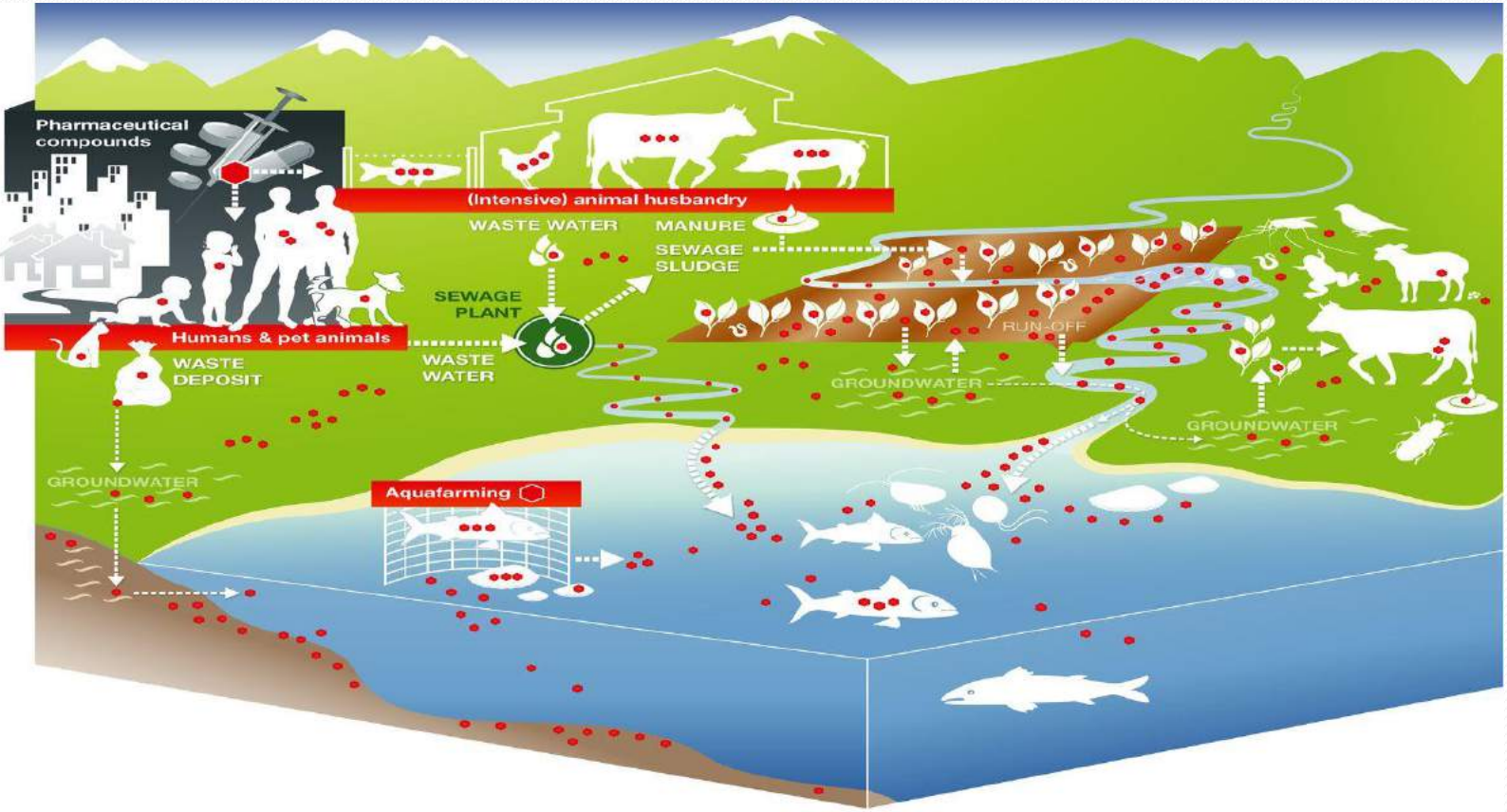


Figure 1. Pathway of antibiotics for human and veterinary use in the environment. Starting with the application of antibiotic agents in human and veterinary medicine in the upper left corner, the spreading of antibiotic residues in the ecosystem is drawnas a web of exposure pathways. The antibiotic residues are symbolically represented as red dots.

Where have all the antibiotics gone?

- Excreta from domestic animals, together with wastewater from cleaning stables, end up in manure storage tanks or lagoons.
- Again, the manure may then be used as fertilizer. The digested residues are also used as fertilizer.
- The consumption of crops—especially raw vegetables from manured soils—exposes humans to microorganisms from the soil and might therefore contribute to the spread of resistance .
- In addition, antibiotics enter the aquatic environment directly from pharmaceutical production facilities

Where have all the antibiotics gone?

- Ciprofloxacin—a commonly prescribed fluorochinolone in human medicine and a transformation product from enrofloxacin, which is used in veterinary medicine.
- Waste-water treatment removes up to 90% of ciprofloxacin by sorption to sewage sludge, but biological degradation is poor.
- As a result, ciprofloxacin accumulates in sewage sludge and, if the sludge is used as fertilizer, in the soil in concentrations in the low mg per kg range.
- *In the soil, ciprofloxacin persists for more than 90 days with only minimal transformation.*
- Although the strong adsorption to soil might reduce its bioavailability, it still elicits effects on soil microorganisms for long periods of time:
- *The resistance gene qnrS was detectable in soil treated with ciprofloxacin from day 14 on*

- The presence of antibiotics in the environment in biologically relevant concentrations has the potential to select for resistant bacteria, archaea, viruses and phages.

- In some cases, selection is not the only mechanism, but also the spread of already resistant microorganisms from WWTPs or manure

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RESEARCH ARTICLE

Mobilizable antibiotic resistance genes are present in dust microbial communities

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Abstract

The decades-long global trend of urbanization has led to a population that spends increasing amounts of time indoors. Exposure to microbes in buildings, and specifically in dust, is thus also increasing, and has been linked to various health outcomes and to antibiotic resistance genes (ARGs). These are most efficiently screened using DNA sequencing, but this method does not determine which microbes are viable, nor does it reveal whether their ARGs can actually disseminate to other microbes. We have thus performed the first study to: 1) examine the potential for ARG dissemination in indoor dust microbial communities, and 2) validate the presence of detected mobile ARGs in viable dust bacteria. Specifically,

Mobilizable antibiotic resistance genes……

- Antibiotics are intensively used and have become widespread in the environment, enriching resistance factors in areas as diverse as hospitals, cattle stool, wastewater treatment plants, and drinking water.
- Much more prevalently, however, *antimicrobial resistance elements can remain resident in otherwise harmless organisms in everyday indoor environments*
- Genes related to antibiotic resistance were also more recently detected in outdoor and indoor dust.
- ARGs in built environments are of particular relevance since humans in urban areas spend ~90% of their lifetime indoors
- This study shows that dust within modern buildings is a reservoir of ARGs and a possible vector for bidirectional transfer of ARGs between the human microbiome and the outdoor environment

- Better knowledge and more information on the fate of antibiotics as well as the development and spread of antibiotic resistance in the environment are required to understand the underlying processes and identify hot spots.
- It is crucial to integrate aspects from human medicine, animal health and environmental considerations.
- Such information eventually informs regulatory policies and legislation to protect human and animal health and the environment.
- The growing problem of multi-drug-resistant major pathogens leaves us little time to act.


THANK
YOU!

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