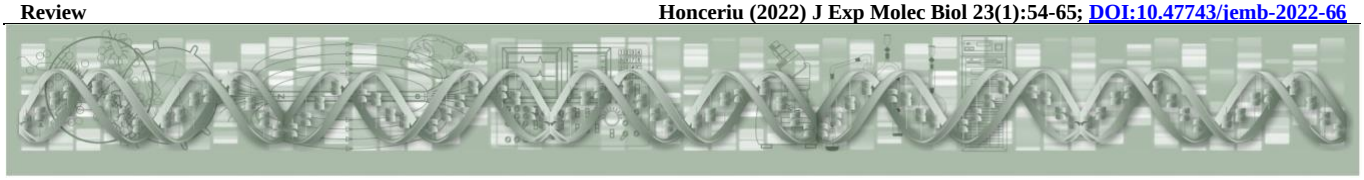

ACQUIRED ANTIBIOTIC RESISTANCE

IASMINA HONCERIU[1*]

[1] Department of Biology, "Alexandru Ioan Cuza" University of Iasi, Iasi 700506, Romania
* honceriu.iasmina@yahoo.com

Abstract

Antibiotics are chemicals used to control bacterial infections, produced by microorganisms (fungi, some bacteria) or obtained synthetically or semi-synthetically. The discovery of antibiotics has revolutionized the medical system, reducing morbidity and mortality rates and improving quality and length of life. Antibiotics affect essential processes in the bacterial cell, but bacteria have adapted to their action by developing antibiotic resistance. The emergence of multidrug-resistant bacteria threatens the healthcare system globally by making it difficult to find effective antibiotics. This review focuses on the mechanisms leading to acquired antibiotic resistance, namely mutations and horizontal gene transfer, and on mobile genetic elements associated with antibiotic resistance (plasmids, transposons, and integrons) that disperse antibiotic resistance genes between bacteria.

Keywords: horizontal gene transfer, multidrug-resistant bacteria, plasmid, transposon, integron

SHORT HISTORY OF ANTIBIOTICS

The use of antibiotics dates from 350-550 AD as analysis of human bone remains from Nubia, Sudan has detected traces of tetracycline embedded in the mineral structure of bone (Bassett E.J. *et al*., 1980). The presence of tetracycline can be explained by the incorporation of products containing it into the diet, which confirms that the Nubian Sudanese population of that time observed the effect of certain products on infections but were unaware of the chemicals they contained. Further evidence of the use of antibiotic substances for treatment is also revealed by anecdotes about the red soils of Jordan, soils used mainly for curing skin infections (Aminov R.I., 2010). The composition of these soils has been analyzed and antibiotic-producing actinomycetes of the polypeptide class have been identified (Sobell H.M., 1985).

The use of antibiotics throughout history may be a factor in the accumulation of genes that give bacteria resistance to antibiotics. The history of these types of genes has been attempted through phylogeny tests, which indicate the presence of genes conferring resistance to several classes of antibiotics in nature long before the "antibiotic era" (Aminov R.I., Mackie R.I., 2007). Thus, it has been established that some β-lactamases, enzymes that can degrade some antibiotics such as penicillins, are thought to be millions of years old (Garau G. *et al*., 2005). It can be said that β-lactamase secreting bacteria are resistant to penicillins.

The first purified antibiotic whose effect on bacteria was known is penicillin. Its potential was suspected by Flemming in 1928 (Fleming A., 1929), from the appearance of growth inhibition zones of *Staphylococcus aureus* colonies in the vicinity of *Penicillium sp.* colonies. Thus, Flemming deduced that *Penicillium sp.* produces substances in the environment that inhibit the growth of *Staphylococcus aureus*. In 1940, penicillin was successfully purified (Chain E. *et al*., 2005), clinical trials were carried out and in 1945 the antibiotic was marketed and used.

So, in 1940 the "antibiotic era" begins, when more and more antibiotics are discovered, such as streptomycin (Schatz A., Waksman S.A., 1944). After 1970, the discovery of new antibiotics greatly decreased. The chemical structure of already known antibiotics started to be modified, resulting in semi-synthetic and chemically synthesized antibiotics (Aminov R.I., 2010). The timeline of antibiotic discovery is presented in **Figure 1**.

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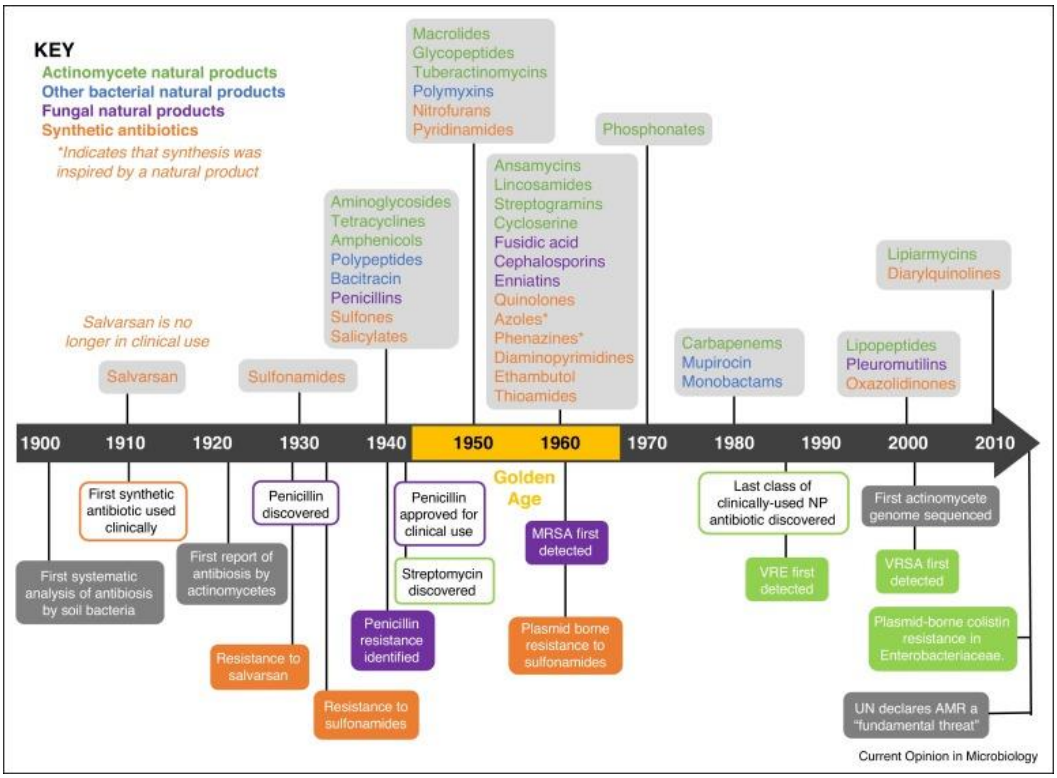

Figure 1: The timeline of antibiotic resistance

Depending on their source, the antibiotics are colored: green – actinomycetes; blue – other bacteria; purple – fungi; orange – synthetic. The first reports of antibiotic resistant species are shown at the bottom of the timeline: MRSA (methicillin-resistant *S. aureus*), VRE (vancomycin-resistant enterococci), VRSA (vancomycin-resistant *S. aureus*), and plasmid-borne colistin resistance in *Enterobacteriaceae.* Source: Hutchings M.I. *et al*, 2019.

With the introduction of antibiotics in the treatment of infectious diseases, it was considered that a potential evolution of antibiotic resistance would be unlikely, based on the assumption that the frequency of mutations leading to resistance would be negligible (Davies J., 1994). Over time, the opposite has proved to be true. No one expected bacteria to adapt to these substances toxic to them and develop a variety of mechanisms to cope and survive. Of all these mechanisms, horizontal gene transfer was the least expected to happen (van Hoek A.H. *et al*., 2011).

Penicillins and aminoglycosides were among the first classes of antibiotics to which bacterial resistance was identified, in 1940 (Abraham, E.P., Chain, E., 1988; Doi Y. *et al*, 2016; Wright G.D., 1999; Bradford P.A., 2001). Over the years, many ecological studies have shown that the use of antibiotics in treatment stimulates the emergence of antibiotic resistance among bacteria belonging to different genera (de Greeff S.C. *et al*., 2020). Some examples of bacteria that have developed resistance are methicillin-resistant *Staphylococcus aureus* (Jevons M.P. *et al*., 1963) and enterobacteria resistant not only to penicillin but also to semi-synthetic antibiotics such as cephalosporins, carbapenems (Kumarasamy K.K. *et al*., 2010). The emergence of multiple antibiotic-resistant bacteria poses a challenge to the healthcare system, increasing morbidity, mortality, and costs due to infections (ECDC/ EMEA Joint Working Group, 2009; Antimicrobial Resistance Collaborators, 2022).

ANTIBIOTICS CLASSIFICATION & MECHANISMS

Antibiotics classification

Depending on their chemical structure, origin, and/or mechanism of action, antibiotics can be grouped into several classes. In **Table 1**, only a few of these classes are presented (Hutchings M.I. *et al*., 2019).

Table 1. Classification of antibiotics (Hutchings M.I. *et al*., 2019):

Class of antibiotics	Names of antibiotics
Penicillins	penicillin G, penicillin V, oxacillin, ampicillin, amoxicillin, mezlocillin
Cephalosporins	cephalothin, cefoxitin, cefixime, cefepime
Carbapenems	imipenem, doripenem
Monobactams	aztreonam

Aminoglycosides	streptomycin, neomycin, kanamycin, gentamicin
Tetracyclines	tetracycline, doxycycline
Macrolides	erythromycin
Sulphonamides	sulfamethoxazole
Quinolones	norfloxacin, ofloxacin, ciprofloxacin
Glycopeptides	vancomycin
Amphenicol	chloramphenicol

Antibiotics mechanisms

Antibiotics act on several levels in the bacterial cell (van Hoek A.H. *et al*., 2011). First, they can inhibit protein synthesis (e.g., aminoglycosides, macrolides, chloramphenicol, and tetracyclines). Aminoglycosides have a broad antimicrobial spectrum and are often used in combination antibiotic therapy. Chloramphenicol has an affinity for the peptidyltransferase of the 50S ribosomal subunit of 70S ribosomes (Schwarz S. *et al*, 2004; van Hoek A.H. *et al*., 2011). Binding to this enzyme prevents peptide chain elongation, thus inhibiting protein synthesis.

Another mechanism of action is that antibiotics can interact with the synthesis of nucleic acids - DNA or RNA (e.g., quinolones). Quinolones inhibit the function of DNA gyrase and topoisomerase IV. These two enzymes are essential for DNA replication, so their disturbance results in the death of the bacteria (Hooper D.C., 2000; van Hoek A.H. *et al*., 2011).

Some antibiotics alter the energy metabolism of a bacterial cell (e.g., sulphonamides). Because of the structural analogy to p-aminobenzoic acid, sulfonamides competitively inhibit the enzyme dihydropteroate synthase (DHPS). P-aminobenzoic acid is part of the biosynthetic pathway leading to folic acid, and the DHPS protein is part of the folate biosynthetic pathway. Folate is necessary for thymine production, so the lack of it stops bacterial growth and division (Roberts M.C., 2002; van Hoek A.H. *et al*., 2011).

Also, some antibiotics destroy the cell wall or inhibit its synthesis (e.g., penicillins, glycopeptides). Penicillins inhibit cell wall synthesis by binding to the bacterial penicillin-binding proteins (PBPs). That way, penicillins interfere with the structural crosslinking of peptidoglycans, thus preventing terminal transpeptidation in the bacterial cell wall. As a result, the cell wall is weakened, resulting in cell death due to osmotic pressure (van Hoek A.H. *et al*., 2011).

Through these mechanisms, antibiotics affect essential processes in the bacterial cell: packaging of the DNA molecule, transcription and translation processes, and biosynthesis of some molecules (Pontes M.H., Groisman E.A., 2020; van Hoek A.H. *et al*., 2011).

ANTIBIOTIC RESISTANCE

Bacteria have impressive genetic plasticity, which allows them to live in the most unfavorable environments, including those containing antibiotics. Bacteria occupying the same ecological niche as certain antibiotic-producing microorganisms (fungi, actinomycetes, other bacteria) will find ways to adapt to these unfavorable conditions by developing resistance mechanisms.

Depending on the involvement or non-involvement of genetic factors, bacterial resistance to antibiotics can be of two types: acquired and non-acquired resistance.

I. Non-acquired resistance results from non-genetic factors and it can either be exhibited by a broad fraction of the bacterial community or by a small subpopulation called persisters (Pontes M.H., Groisman E.A., 2020). Non-acquired antibiotic resistance has been attributed to the activity of toxins part of toxin-antitoxin systems, signaling molecules (such as ppGpp and pppGpp - guanosine (penta) tetraphosphate), and ATP. Nonetheless, these molecules are dispensable for non-acquired antibiotic resistance. On the other side, factors such as nutrient limitation in the environment, bacterial exposure to bacteriostatic substances, or the expression of genes that slow bacterial cell growth are essential in the non-acquired resistance phenomenon (Pontes M.H., Groisman E.A., 2020). Antibiotic persistence leads to cell growth inhibition as a protective measure against antibiotics.

II. Acquired resistance can be achieved by two mechanisms: by mutations in genes associated with the mechanism of action of a specific antibiotic or by the acquisition of DNA from other bacteria of the same or different species or genera through horizontal gene transfer (Munita J.M., Arias C.A., 2021).

II.1. Resistance acquired through mutations

Mutations that lead to the development of antibiotic resistance alter the action of the antibiotic through the following mechanisms (Munita J.M., Arias C.A., 2021):

II.1.1. changes in the target of a particular antibiotic, thus decreasing affinity. Modification of target structures can be achieved by point mutations in the genes coding for the target structures or by enzymatic modifications of the binding sites (e.g. by

methylation). For example, a deletion or insertion (reading frame modification) within the *ddl* gene can lead to glycopeptide class resistance (Casadewall B., Courvalin P., 1999). Single nucleotide polymorphisms (SNPs) can lead to resistance to synthetic antibiotics such as quinolones or sulfonamides (Hooper D.C., 2000; Ruiz J., 2003).

II.1.2. reducing the amount of antibiotics taken up from the external environment - by decreasing the permeability of the outer membrane, which reduces the access of antibiotics inside the cell. The outer membrane is the first line of defense against antibiotics and can prevent their penetration at the intracellular level via channels called porins (Pagès J.M. *et al*., 2008). The most affected are hydrophilic antibiotics: penicillins, and tetracyclines, whose penetration of the outer membrane will be blocked.

II.1.3. pumping of the antibiotic out of the cell by active efflux, resulting in a concentration of antibiotic in the cytoplasm that is too low to be able to act. Efflux pumps are present in both Gram-positive and Gram-negative bacteria and are of two types: substrate-specific and broad substrate specificity (Munita J.M., Arias C.A., 2016). The former is specific to a particular antibiotic, such as the *mef* genes for macrolides in pneumococci. The last is usually found in multidrug-resistant bacteria (Poole K., 2005). These mechanisms affect the action of a wide range of antibiotics classes such as carbapenems, β-lactams, and fluoroquinolones (Munita J.M., Arias C.A., 2016).

II.1.4. alteration of metabolic pathways. Inactivation of several genes involved in basic metabolic processes alters bacteria's susceptibility to antibiotics. Metabolic mutations arise in response to antibiotic treatment. For example, mutation in the 2-oxoglutarate dehydrogenase (*sucA*) enzyme in an *E. coli* strain leads to lower basal respiration which prevents antibiotic-mediated induction of tricarboxylic acid cycle activity, thereby minimizing lethality and avoiding metabolic toxicity (Lopatkin A.J. *et al*, 2021).

II.2. Resistance acquired by horizontal gene transfer

Horizontal gene transfer is the process by which genetic material is transferred between bacteria belonging to the same generation, the same species, or different species. Horizontal gene transfer adds new genetic variation to avoid the destructive effect of the gradual accumulation of point mutations.

Horizontal gene transfer is a crucial factor in bacterial evolution. Acquisition of external genetic material can be achieved by four main methods (Liu Y. *et al*., 2020) (**Fig. 2**):

II.2.1. conjugation: via mobile elements or by direct transfer from one bacterium to another (Manson J.M. *et al*., 2010);

II.2.2. transformation (the incorporation of DNA uncovered by a membrane);

II.2.3. transduction (via phages);

II.2.4. vesiduction: via extracellular vesicles.

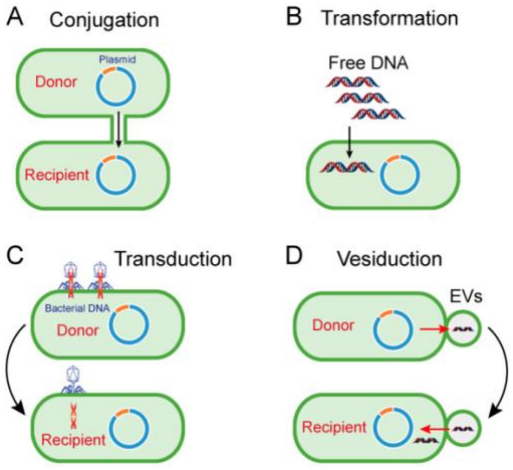

Figure 2: Conjugation, transformation, transduction, and vesiduction - the four mechanisms by which horizontal gene transfer takes place

A. Conjugation involves the transfer of genetic material from the donor cell to a recipient cell. In this case, a plasmid has been transferred in which a resistance gene, marked orange, is found; **B**. Transformation involves the uptake and integration of free DNA molecules; **C.** Transduction involves the transfer of bacterial DNA via bacteriophages; **D.** Vesiduction refers to the transfer of extracellular vesicles (EVs) containing genetic material between two bacterial cells. Source: Liu Y. *et al*., 2020.

II.2.1. Conjugation

Conjugation allows the transfer of genetic material between two bacterial cells in the same environment. Conjugation can be achieved in two different ways: in Gram-negative bacteria, the transfer of genetic material is via the pilus, and in Gram-positive

bacteria, the transfer is via surface adhesion (Kohler V. *et al*., 2019). With this method, a safer transfer is achieved as it ensures the protection of the genetic material from the external environment. Conjugation allows the transfer of resistance genes between bacteria with very large phylogenetic distances (Tamminem M. *et al*., 2012), which suggests the remarkable role that conjugation has played in the dissemination of resistance genes from different gene pools. For example, resistance genes to ticarcillin and kanamycin are transferred through conjugation between two *Acinetobacter baumanii* strains, namely NU013R and NU015R, via the plasmid group GR6 (Leungtongkam U. *et al*, 2018). A study done by Talreja D. *et al* in 2014 indicates that resistance genes for tetracycline and quinolone can be transferred through conjugation from *A. baumanii* (isolate AB12) to *E. coli* J53.

II.2.2. Transformation

Transformation is the process by which free extracellular DNA fragments are taken up and integrated into the genome of some bacteria either in the chromosome or incorporated as plasmids. Bacterial genera capable of transformation include *Acinetobacter* (resistant to ampicillin and tetracycline), *Neisseria, Haemophilus, Pseudomonas* (the pKUM plasmid confers resistance to the third generation of cephalosporins, aminoglycosides, and cefepime in *P. aeruginosa*) (Jafar E. *et al*, 2013), *Staphylococcus (mecA* gene confers resistance to β-lactam antibiotics*)* (Maree M. *et al*, 2022), and *Streptococcus* (Johnston C. *et al*., 2014; Traglia G.M. *et al*., 2014).

A study by Sturød K. *et al*. in 2018 found that under stressful conditions such as exposure to antibiotics, transformation can be stimulated. Transformation is also stimulated within biofilms, as was shown by Nadell C.D. *et al*. in 2009 and by Seitz P. and Blokesch M. in 2013.

II.2.3. Transduction

Transduction is carried out by bacteriophages. In this process, genes from the donor (host) cell are first incorporated into the bacteriophage genome. Next, the bacteriophage will inject the genes from the donor cell into the recipient cell. The transfer of DNA via bacteriophages protects it from the action of environmental DN-ases and physical factors that could degrade it (Liu Y. *et al*., 2020).

Transduction has been shown to play an important role in the transfer of antibiotic resistance genes (Rolain J.M. *et al*., 2011; Quirós P. *et al*., 2014). For example, genes for tetracycline and penicillin resistance in *S. aureus* populations can be transmitted through transduction (Mašlaňová, I. *et al*, 2016). In hospitals, the transfer of genes coding for β-lactamases by transduction between different *Pseudomonas* strains (Blahová J. *et al*., 2000) or between *Acinetobacter* strains has been recorded (Krahn T. *et al*., 2016).

Some bacteriophages can transmit DNA fragments of sizes larger than 100 kb (Ochman H. *et al*., 2000), a size sufficient to carry plasmids. For example, transduction of a 5667 bp plasmid with tetracycline and aminoglycoside resistance genes between different strains of *S. aureus* has been achieved (Zeman M. *et al*., 2017). Transduction between different genera has also been recorded: a 5620 bp plasmid with a kanamycin resistance gene between *Serratia* and *Kluyvera* genera via the bacteriophage ϕMAM1 (Matilla M.A., Salmond G.P.C., 2014).

II.2.4. Vesiduction

In addition to the above three mechanisms a new horizontal gene transfer mechanism called vesiduction has recently been discovered (Soler N., Forterre P., 2020). By this, the donor bacterium secretes a vesicle from its membrane inside which genetic material is contained. The existence of a vesicle protects the DNA fragment from the action of external environmental factors such as DN-ases, restriction enzymes, or physical or chemical factors (Liu Y. *et al*., 2020). Through these extracellular vesicles, plasmids can be transferred from one cell to another (Erdmann S. *et al*., 2017). The precise mechanism by which vesiduction is achieved is still unknown; how the vesicle is secreted and how the vesicle is recognized and incorporated by fusion by the recipient cell is still unknown (Liu Y. *et al*., 2020).

Transformation is probably the simplest form of horizontal transfer, but only a few medically relevant bacterial species can naturally incorporate foreign DNA molecules uncoated by a membrane to develop resistance (Munita J.M., Arias C.A., 2021). In these species, resistance arises mainly from conjugation, which involves contact between two cells. Conjugation occurs mainly in the human gastrointestinal tract during antibiotic administration (Munita J.M., Arias C.A., 2021). The most important mobile elements by which conjugation takes place are plasmids, transposons, and integrons (Munita J.M., Arias C.A., 2021), with a key role in the development and dissemination of genes responsible for antibiotic resistance among clinically important bacterial species.

MOBILE GENETIC ELEMENTS ASSOCIATED WITH ANTIBIOTIC RESISTANCE

Plasmids

Plasmids are extrachromosomal, double-stranded DNA molecules capable of self-replication independent of the bacterial chromosome. They do not contain genes essential for bacterial survival but have more accessory genes, such as those conferring

antibiotic resistance (Girlich D. *et al*., 2020). The persistence of plasmids is enhanced when they contain genes useful to the host cell, such as genes for resistance during antibiotic contact. Many resistance genes circulate between bacteria via plasmids (Blair J.M.A. *et al*., 2015). Plasmids spread in bacterial populations mainly by conjugation.

An example of a resistance gene that is transmitted via plasmids is the gene coding for the enzyme β-lactamase. Through the conjugation process, this gene is transmitted intra- and interspecifically in bacterial genera such as *Pseudomonas, Enterobacteriaceae*, and *Acinetobacter* (Gorrie, C.L. *et al*., 2018; Phan H.T.T. *et al*., 2018; Martin J. *et al*., 2017).

Plasmid transfer leads to the emergence of methicillin-resistant *Staphylococcus aureus* (MRSA) tough to treat with conventional antibiotics (Lerminiaux, N.A., Cameron, A.D.S., 2018). MRSA is a frequent cause of nosocomial infections. In MRSA infections, vancomycin is a last resort. However, due to the horizontal transfer of a plasmid derived from *Enterococcus faecalis* containing a vancomycin resistance gene (Gardete S., Tomasz A., 2014) to the MRSA genome, MRSA transformed into vancomycin-resistant *Staphylococcus aureus* (VRSA). That is a typical example of how, through horizontal gene transfer, multiple resistance genes accumulate within the genome, leading to the emergence of multidrug-resistant bacteria for which it is difficult to find an effective antibiotic.

Antibiotic administration stimulates conjugation. For example, exposure to minimal inhibitory concentrations in combination treatment with kanamycin and streptomycin stimulates conjugation in Gram-negatives such as *E. coli (*Zhang P.Y. *et al*, 2013). After antibiotic therapy, expression of *oppA* and *rbsB* rose, leading to enhanced transmission of conjugation plasmids and bacterial survival.

Insertion sequences and transposons

An insertion sequence is a small transposable element composed of one or two genes with a role in encoding the transposase enzyme, flanked by inverted repetitive sequences of various lengths (**Fig. 3a**). A transposon consists of a DNA fragment (which may contain a gene for antibiotic resistance) flanked by an insertion sequence, as shown in **Fig. 3b**. The transposase enzyme recognizes the repetitive sequences to which it attaches and cuts the DNA, then moves it to another site. Many transposons have a promoter upstream of the coding genes (e.g. upstream of resistance genes) with which expression of the gene(s) is achieved (Girlich D. *et al*., 2020).

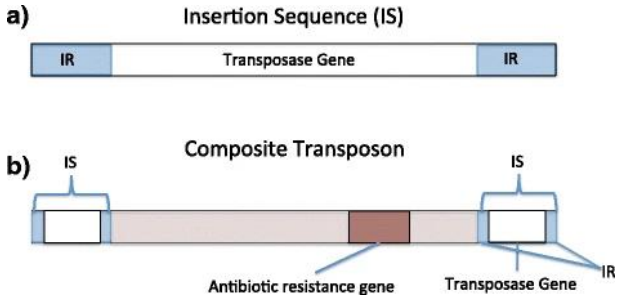

Figure 3. Structure of an insertion sequence (IS) (a) and a composite transposon (b).

a): the transposase coding gene is flanked by two inverted repeat sequences (IR); **b):** the structure of a composite transposon: in the middle is the DNA sequence to be passed to another bacterial cell. Included in this DNA sequence is a resistance gene. The DNA sequence of interest is flanked on each side by an insertion sequence (IS). Source: Warnke J., Ali H.H., 2016.

Transposons are DNA sequences that can move within the genome. They can have both a positive and a negative role on the genome: although they play a very important role in bacterial evolution, their insertion within a coding region of a gene (CDS, CoDing Sequence) can lead to the inactivation of the gene within which they have integrated (Girlich D. *et al*., 2020).

Transposons play an important role in the transmission of accessory structures, such as some metabolic pathways or resistance genes. For example, one strain of enterococcus resistant to several antibiotics, including vancomycin, is *E. faecium* strain C68, isolated in Ohio, which is resistant to at least 11 antibiotics belonging to 7 different classes (Rice L.B. *et al.*, 2010). Two of the resistance genes were found to be situated within transposons: on transposon *Tn916* is found the *tetM* gene, conferring tetracycline resistance, and on transposon *Tn1549* (**Fig. 4**) is the *vanB* gene, conferring vancomycin resistance. The *Tn916* transposon and the *Tn1549* transposon are part of the same family: the *Tn916* family (Lambertsen L. *et al*., 2017). These two transposons can transfer resistance genes from one bacterium to another, as observed by Rice L.B. *et al*. in 2010.

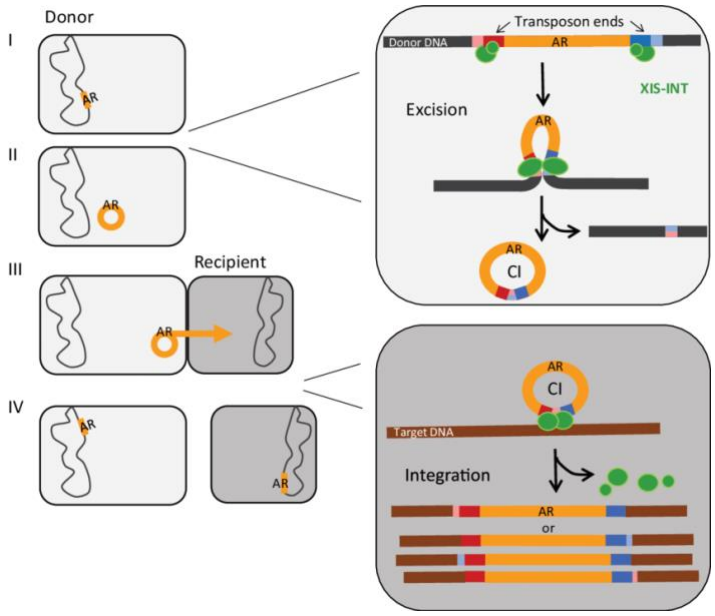

Figure 4. Movement of *Tn916* family transposons.

I: the chromosome of the donor cell holds the conjugative transposon (yellow) containing an antibiotic resistance gene (AR); **II:** the transposon is excised from the chromosome, gaining a circular shape (CI). Details of excision are shown in the top insert. In the structure of the transposon, you can see marked yellow the DNA fragment to be transferred - in this case, an antibiotic resistance gene. This gene is flanked by a dark red and a dark blue portion representing the ends of the transposon. The ends of the transposon are flanked by a light red and a light blue colored portion that are essential for efficient and accurate transposition. The chromosomal DNA of the donor bacterium is stained black. The XIS-INT proteins (XIS integrin - INT protein complex) (shown in green) recombine the transposon ends forming a CI, thus the donor DNA is released; **III:** transposon transfer between two bacteria by conjugation process; **IV:** with the help of the INT protein, the CI is integrated into the donor cell chromosome. The bottom insert presents the integration of the transposon into the chromosome of the recipient cell. Upon integration of the CI into the target DNA, there are four possible integrated versions carrying the right or left flanking DNA (light blue and light red). The chromosomal DNA of the recipient cell is stained brown. Source: Lambertsen L. *et al.* 2017.

By recombining the red and light blue stained portions, excision of the transposon from its original location occurs. Integration of the transposon into the DNA of the recipient bacterial cell is also achieved by recombination of the two portions colored red and light blue.

Transposon recombination is catalyzed by the enzyme integrase (INT). The integrase recognizes the specific repetitive sequences in the transposon ends (colored dark red and blue) to which it attaches, then recombines them to form a circular-shaped transposon (CI). Since integrase must perform both excision and transposon integration, another enzyme will assist in the excision process: the XIS protein (Lambertsen L. *et al*., 2017). The transposon contains the genes required for the synthesis of INT and XIS proteins.

For excision to take place, the two enzymes will form the XIS-INT complex. Once the transposon has been integrated, the complex will separate and detach from the DNA fragment.

The *Tn1549* transposon has also been identified in the *Clostridium*, *Eggerthella*, *Atopobium*, *Ruminococcus*, and *Streptococcus* genera (Marvaud J.C. *et al*., 2011; Dehoux P. *et al*., 2016). Transfer of the Tn1549 transposon has been observed to occur from *Clostridium symbiosum* to *E. faecium* and *E. faecalis* (Launay A. *et al*., 2006).

If a plasmid has arrived within a bacterial cell, either by conjugation or transduction, transposons can further mobilize genes for resistance within the newly arrived plasmid by copying these genes onto a new plasmid or into the bacterial chromosome (Conlan S. *et al*., 2014; Mulvey M.R. *et al*., 2016; Ludden C. *et al*., 2017). Through this mechanism, transposons create a new dynamic in which resistance genes taken from a newly acquired plasmid will be able to be transferred to other plasmids, thereby increasing the potential for resistance genes to be transmitted to other bacteria through the conjugation process.

Integrons

Integrons are mobile DNA fragments with a major role in bacterial adaptation and evolution. Integrons are often located on plasmids or transposons, thus making their horizontal transfer possible as they cannot be transmitted by themselves.

Integrons contain three essential components: a gene encoding the integrase enzyme (*intI* gene), which catalyzes the incorporation of the integron from the original cell into a new bacterial cell; the *attI* recombination site (recognized by the integrase enzyme; helps identify the site for gene integration); the integrated cassettes composed of a gene and the *attC* site (imperfect inverted repeat, a palindrome of variable length and sequence; *attC* serves as a recombination site for *IntI* enzymes), located downstream of the gene and a promoter (*Pc*) that enables the transcription of the genes in the integron structure (Akrami F. *et al*., 2019; Larouche A., Roy P.H., 2011). These components allow the integron to acquire new genes (gene cassettes), such as antibiotic resistance genes.

How integrons work is shown in **Figure 5**:

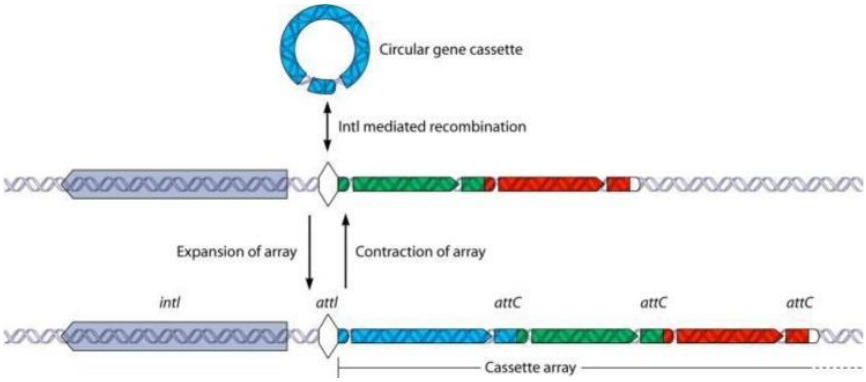

Figure 5. Gene acquisition by integrons.

Integrons include genes based on specific recombination between *attC* in the circular gene cassette and *attI* in the integron. New genes will insert close to the *intI* gene and promoter. The integron can increase in size by incorporating new genes, or it can shrink by excising genes in the form of a circular gene cassette. *IntI:* integrase enzyme gene; *attI*: recombination site recognized by the integrase enzyme; *attC*: imperfect inverted repeat, recombination site for IntI enzymes. Source: Akrami F. *et al*, 2019.

Integrons are divided into five groups based on phylogenetic similarities of 16S rRNA and *IntI* gene sequence. Thus, integron classes I, II, III, IV, and V have been identified. Integrons are more commonly found in Gram-negative bacteria (Akrami F. *et al*., 2019).

For example, class I integrons contain resistance genes such as the *sul1* gene (confers resistance to sulphonamides) and enzymes that modify the structure of aminoglycosides. Class I is widespread among *Acinetobacter*, *Pseudomonas*, *Salmonella*, *and Klebsiella* genera (Nandi S. *et al*., 2004). Class III integrons are transmitted by the *Tn402* transposon. Within this class, the *blaGES-1* gene (an extended-spectrum β-lactamase) is found within the IncQ plasmid in *E.coli*. Integron class III also contains the *aacA4* gene, which confers resistance to tobramycin. Class IV contains resistance genes for chloramphenicol and fosfomycin (Deylam Salehi M. *et al*., 2017; Gillings M.R., 2014).

II.3. Horizontal transfer of resistance genes obtained by mutations

As described above, acquired resistance can also be achieved through mutations. Point mutations of a particular gene lead to cell survival because that gene will no longer represent a target for the specific antibiotic. The point mutation removes the interaction with the antibiotic and at the same time preserves the function of the gene, especially if the gene is essential in the life of the bacteria. An example of such a situation is the point mutation of the essential gene *gyrA*, which encodes DNA gyrase. Through this mutation, the bacterium acquires resistance to quinolone and nalidixic acid (Hooper D.C., 2001). In this way, the *gyrA* gene can become a resistance gene, even though it is essentially a gene coding for DNA gyrase, which separates the two DNA strands in the replication process. Such genes are also called resistance alleles (Lerminiaux N.A., Cameron A.D.S., 2018). Resistance genes obtained by mutations of target genes can be transferred between primary bacterial cells by transduction and transformation. Horizontal transfer of resistance alleles most commonly occurs between genetically related individuals as allele exchange is based on homology (Lerminiaux N.A., Cameron A.D.S., 2018).

Quinolone resistance is an example of how multiple modes of horizontal transfer can contribute to and create antibiotic resistance. Of the quinolones, ciprofloxacin, and norfloxacin are of particular clinical importance, but their success results in environmental pollution as these antibiotics have been detected in wastewater discharged from hospitals (Ory J. *et al*., 2016; Szczepanowski R. *et al.*, 2008). Quinolone resistance is increasing, a phenomenon observed particularly in bacteria of the genera *Pseudomonas*, *Clostridium*, *Acinetobacter*, and *Enterobacteriaceae* (Dalhoff A., 2012). The mechanism of action of this class of antibiotics is the targeting of DNA topoisomerases. To protect against this effect, mutations occur within the gene encoding a topoisomerase called DNA gyrase. The mutation produces changes in the amino acid sequence within the enzyme, thus reducing the affinity of the antibiotic (Lerminiaux N.A., Cameron A.D.S., 2018).

It has been demonstrated that quinolones can enhance the transformation process in *Streptococcus pneumoniae* (Prudhomme M. *et al.*, 2006). In conclusion, exposure to quinolone antibiotics increases the rate of horizontal gene transfer and the acquisition of resistance alleles.

Plasmid-mediated quinolone resistance is mediated by *qnr* genes (Jacoby G. *et al.*, 2008). These *qnr* genes can associate with genes for penicillin resistance or with plasmids on which several resistance genes for different antibodies are found. These associations have been observed in bacterial genera such as *Escherichia, Acinetobacter*, and *Klebsiella* (Conlan S. *et al.*, 2014; Sana F. *et al.*, 2014; Ludden C. *et al.*, 2017; Lewis T. *et al*., 2010; Paterson D.L., Bonomo R.A., 2005).

In addition to transformation and transduction mechanisms, resistance genes within plasmids can be transmitted from one bacterium to another by conjugation (Sana F. *et al.*, 2014; Osei Sekyere J., Amoako D.G., 2017). Transformation and transduction are more valid for the transmission of resistance alleles, such as mutations in DNA gyrase, and the transmission of these mutations can be achieved efficiently via plasmids.

CONCLUSIONS

Antibiotics are one of the greatest discoveries in human history to combat bacterial infections. However, bacteria developed antibiotic resistance mechanisms to resist their toxicity. Behind their pathogenic effect, bacteria developing antibiotic resistance should be viewed as a "normal" adaptive response in the evolution course (Munita J.M., Arias C.A., 2016). Although initially unexpected, antibiotic resistance became a rapidly evolving phenomenon, now in the 21st century representing one of the greatest threats globally in clinical settings, even more with the apparition of multidrug-resistant bacteria.

There are two known types of resistance: acquired and non-acquired antibiotic resistance (Pontes M.H., Groisman E.A., 2020; Munita J.M., Arias C.A., 2021). Acquired resistance can be achieved by mutations of genes associated with the action of an antibiotic (especially point mutations, leading to the emergence of resistance alleles) or by horizontal gene transfer (Munita J.M., Arias C.A., 2021). Horizontal gene transfer can be achieved by conjugation, transformation, transduction, and vesiduction, of which vesiduction was recently discovered (Liu Y. *et al*., 2020) yet incompletely understood. Steady efforts to study antibiotic resistance mechanisms are required (Munita J.M., Arias C.A., 2016).

Acquired antibiotic resistance is just a part of the entire antibiotic resistance phenomenon, but an important one as horizontal gene transfer can cause the emergence of multiple antibiotic-resistant bacteria through the accumulation of multiple resistance genes between different species and genera, as in the case of methicillin-resistant *Staphylococcus aureus* (MRSA) (Lerminiaux, N.A., Cameron, A.D.S., 2018) and vancomycin-resistant *Staphylococcus aureus* (VRSA) (Gardete S., Tomasz A., 2014). We are currently in need of new antibiotics and treatments to control bacterial infections.

Understanding the genetic basis of antibiotic resistance is essential in creating new antibiotics and developing new innovative treatment strategies to stop the emergence and spread of antibiotic resistance, especially amongst multidrug-resistant bacteria. Regardless of the type of treatment to be developed, we should keep in mind the probability that bacteria will eventually adapt and develop mechanisms to survive, as is natural. This "fight" will be a tortuous, challenging, and circular process.

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