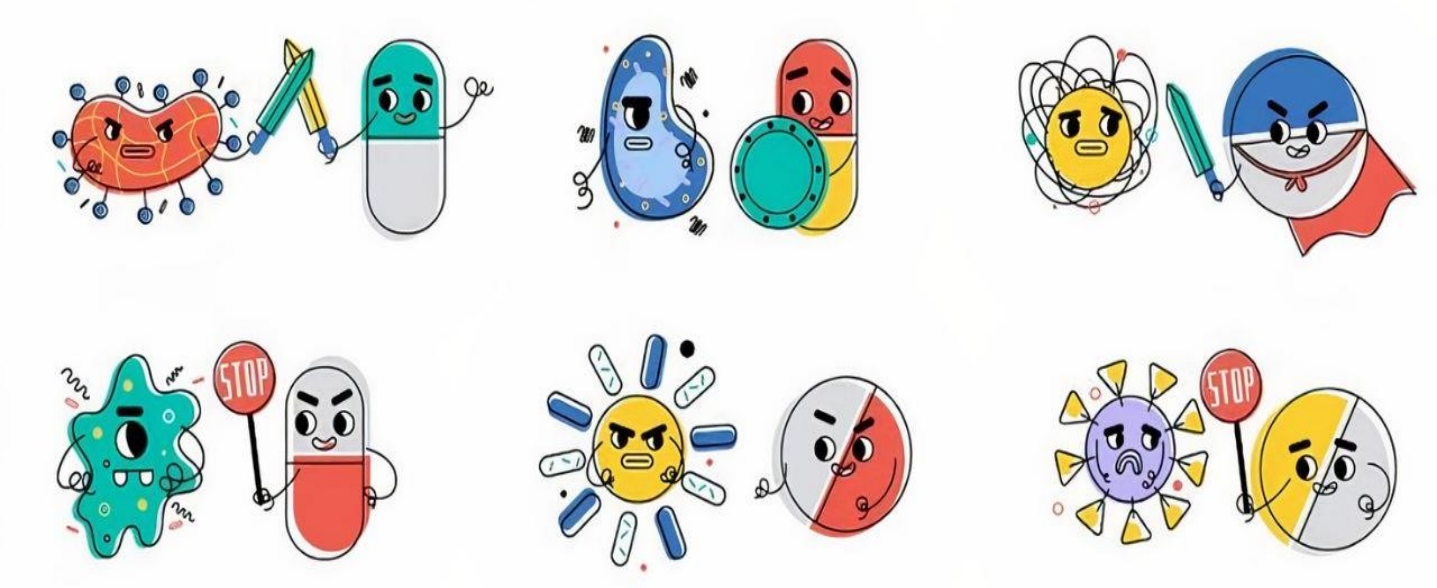
AN OVERVIEW OF THE ANTIMICROBIAL RESISTANCE: CLASSIFICATION, MODES OF ACTION AND MECHANISMS



Republic of Iraq

Ministry of Higher Education and Scientific Research

University of Babylon

College of Science for Women

AN OVERVIEW OF THE ANTIMICROBIAL RESISTANCE: CLASSIFICATION, MODES OF ACTION AND MECHANISMS

A Review Submitted to The Council of the College of Science for Women and the Committee of the undergraduate Studies of University of Babylon In partial fulfillment of the Requirements for the Degree of B.Sc. Biology – Microbiology

By

Zahraa Mousa Kadhim

Zahraa Hadi Wannas

Supervised by

Prof. Dr. Hasanain Khaleel Ibrahim

2023 A.D. **1444 A.H.**

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

﴿اقْرَأْ بِاسْمِ رَبِّكَ الَّذِي خَلَقَ ۝ خَلَقَ الْإِنْسَانَ مِنْ عَلَقٍ ۝ اقْرَأْ وَرَبُّكَ الْأَكْرَمُ ۝
الَّذِي عَلَّمَ بِالْقَلَمِ ۝ عَلَّمَ الْإِنْسَانَ مَا لَمْ يَعْلَمْ ۝﴾

صَدَقَ اللهُ العَلِيُّ العَظِيمُ

سورة العلق : الآية (1–5)

SUPERVISOR CERTIFICATION

I certify that the preparation of this review entitle"…….. "was prepared by …… …… ,Was made under my supervision at the Department of Biology, College of Science for Women, University of Babylon in partial fulfillment of the Requirements for the Degree of Bachelor of Science in Biology- Microbiology.

Signature:

Supervisor name:

Date:

DEDICATION

We would Dedicated this work to our Family the first and the biggest support

To the people who be there for us.

To our Supervisor for His Guidance.

And last but not least...

To each other’s for macking this 4 years the best years ever.

Zahraa M. & Zahraa H.

ACKNOWLEDGMENTS

I would like to thank God, for letting me through all the difficulties I have experienced your guidance day by day.

I would like to acknowledge and give my warmest thanks to my supervisor (Prof. Dr. Hasanain Khaleel Ibrahim) who made this work possible. His guidance and advice carried me through all the stages of writing my research.

Finally, I would also like to give special thanks to my family as a whole for their continuous support and understanding when undertaking my research. Their prayer for me was what sustained me this far.

TABLE OF CONTENTS

No. item	Title	No. page
	Dedication	I
	Acknowledgments	II
	Table of Content	III-IV
	List of Figures	V
	List of Abbreviations	VI
1.	**Antibiotic**	**1**
1.1	What is the term of antibiotic?	1
1.2.	History of Antibiotics	1
1.3.	The First Antibiotics	2
2.	**Classification of Antibiotics**	**3**
2.1.	β-lactam	3
2.1.1.	Penicillin	4
2.1.2.	Cephalosporin	5
2.2.	Aminoglycoside	6
2.3.	Tetracycline	7
2.4.	Macrolides	8
2.5.	Quinolones	9
2.6.	Sulphonamides	10
3.	**Mechanisms of Action**	**12**
3.1.	Basic Anatomy of Bacterial Cell	12
3.2.	How do antibiotics work?	13
3.3.	Classification of Antibiotics on the Basis of Mechanism of Action	14
3.3.1.	Antibiotics targeting cell wall	14
3.3.2.	Inhibitors of protein biosynthesis	15
3.3.3.	Inhibitors of DNA replication	17
3.3.4.	Folic acid metabolism inhibitors	17
4.	**Mechanisms of Resistance to Antibiotic**	**18**
4.1.	The main four types of resistance to antibiotics develops	18
4.1.1.	Natural resistance	18
4.1.2.	Acquired resistance	19
4.1.2.1.	Chromosomal resistance	19
4.1.2.2.	Extra chromosomal resistance	19

4.1.3.	Cross resistance	20
4.1.4.	Multi-drug resistance and pan-resistance	20
4.2.	Mechanisms of resistance	21
4.2.1.	Limiting drug uptake	22
4.2.2.	Modification of drug targets	24
4.2.3.	Drug inactivation	26
4.2.4.	Drug efflux	27
5.	**MDR, XDR and PDR**	**29**
5.1.	MDR	30
5.2.	XDR	30
5.3.	PDR	30
6.	**Technique and method used to determine the antibiotic resistance**	**32**
6.1.	Molecular techniques	33
6.2.	Microarrays	34
6.3.	Commercial antibiogram methods	34
6.4.	Imaging methods	35
6.5.	Bacterial lysis methods	35
7.	**Antibiotic and Immunity**	**36**
7.1.	Bactericidal vs Bacteriostatic the context of Innate Immunity	37
7.2.	Counteracting the negative effect of antibiotics on immunity	38
8.	**Antibiotic Prophylaxis**	**39**
8.1.	What is antibiotic prophylaxis?	39
9.	**Antibiotic and Vaccine**	**41**
9.1.	Vaccine	41
9.2.	What’s the Difference between Antibiotics and Vaccines?	41
9.3.	Direct and indirect effects of vaccines on antimicrobial resistance	42
9.4.	What makes vaccines more effective?	45
9.5.	Pathways by which vaccines can reduce AMR	46
10.	**Antibiotic vs Probiotic**	**49**
10.1.	Probiotic	49
10.2.	Era of Probiotics	50
10.3.	Combination of Antibiotics and Probiotics	52
10.4.	Use of Probiotics and Their Impact on Microbiota in Infection Diseases	53
	References	**54**
	Antibiotic in Arabic	**67**

LIST OF FIGURES

No. item	Name	No. Page
2.1.	Chemical structure of a beta-lactam ring	3
2.2.	Chemical structure of beta-lactam structure.	5
2.3.	Structure of Aminoglycoside (Streptomycin)	6
2.4.	Structure of Tetracycline	7
2.5.	Structure of Macrolide	8
2.6.	Structure of Quinolone	10
2.7.	Structure of Sulphonamides	11
3.1.	Structure of bacterial cell envelope	12
3.2.	Mechanism of Action of Antibiotics	13
3.3.	Mechanism of action of β lactam antibiotics	15
4.1.	General antimicrobial resistance mechanisms.	22
4.2.	General structure of main efflux pump families	28
5.	Diagram showing the relationship of MDR, XDR and PDR to each other	30
9.1.	Effects of vaccines on antimicrobial resistance	44
9.2.	The usage of antibiotics selects for resistance	45
10.	Mechanisms of action of probiotics	51

LIST OF ABBREVIATION

Abbreviations	Key
PBP	Penicillin-binding protein
PABA	P-aminobenzoic acid
OM	Outer membrane
AG	Aminoglycosides
FQ	Fluoroquinolones
MDR	Multi drug resistance
XDR	Extensive drug resistant
PDR	Pan drug resistant
DHPS	Dihydropteroate synthase
DHFR	Dihydrofolate reductase
MATE	Multidrug and toxic compound extrusion
ABC	ATP-binding cassette
SMR	Small multidrug resistance
MFS	Major facilitator superfamily
RND	Resistance-nodulation-cell division
OMP	Outer membrane protein
MDROs	Multidrug-resistant organisms
GNB	Gram-negative bacteria
MTB	*Mycobacterium tuberculosis*
AMR	Antimicrobial Resistance
ARGs	Antimicrobial resistant genes
RSV	Respiratory syncytial virus

1. Antibiotic

1.1. What is the term of antibiotic?

The term antibiotic was coined from the word „antibiosis" which literally means „against life". In the past, antibiotics were considered to be organic compounds produced by one microorganism which are toxic to other microorganisms (Russell, 2004). As a result of this notion, an antibiotic was originally, broadly defined as a substance, produced by one microorganism (Denyer *et al*., 2004), or of biological origin (Schlegel, 2003) which at low concentrations can inhibit the growth of, or are lethal to other microorganisms (Russell, 2004). However, this definition has been modified in modern times, to include antimicrobials that are also produced partly or wholly through synthetic means. Whilst some antibiotics are able to completely kill other bacteria, some are only able to inhibit their growth. Those that kill bacteria are termed bactericidal while those that inhibit bacterial growth are termed bacteriostatic (Walsh, 2003). Although antibiotic generally refers to antibacterial, antibiotic compounds are differentiated as antibacterial, antifungals and antivirals to reflect the group of microorganisms they antagonize (Brooks *et al*., 2004; Russell, 2004).

Antibiotics came into worldwide prominence with the introduction of penicillin in 1941. Since then they have revolutionized the treatment of bacterial infections in humans and other animals. They are, however, ineffective against viruses.

1.2. History of Antibiotics

The first known use of antibiotics was by the ancient Chinese over 2,500 years ago. Chinese have discovered the therapeutic properties of moldy soybeans and used this substance to cure furuncles (pimples), carbuncles and similar infections. Many other ancient civilizations, including ancient Egyptians and ancient Greeks already used

molds and plants to treat infections due to the production of antibiotic substances from these organisms. But at that time, compounds that develop antibiotic action was unknown (Konstantopoulou A., 2016).

1.3. The First Antibiotics

Penicillin was the first antibiotic discovered in September 1928 by an English Bacteriologist, late Sir Alexander Fleming who accidentally obtained the antibiotic from a soil inhabiting fungus *Penicillium notatum* but its discovery was first reported in 1929 (Aminov, 2010), and clinical trials first conducted on humans in 1940 (Schlegel, 2003; Russell, 2004).In 1928, Fleming began a series of experiments involving the common staphylococcal bacteria. An uncovered Petri dish sitting next to an open window became contaminated with mold spores. Fleming observed that the bacteria in proximity to the mold colonies were dying, as evidenced by the dissolving and clearing of the surrounding agar gel. He was able to isolate the mold and identified it as a member of the *Penicillium* genus. He found it to be effective against all Gram-positive pathogens, which are responsible for diseases such as scarlet fever, pneumonia, gonorrhea, meningitis and diphtheria. He discerned that it was not the mold itself but some 'juice' it had produced that had killed the bacteria. He named the 'mold juice' penicillin. Later, he would say: "*When I woke up just after dawn on September 28, 1928, I certainly didn't plan to revolutionize all medicine by discovering the world's first antibiotic, or bacteria killer. But I suppose that was exactly what I did*."(*Fleming: discoverer of penicillin)*Although Fleming published the discovery of penicillin in the British Journal of Experimental Pathology in 1929, the scientific community greeted his work with little initial enthusiasm. Additionally, Fleming found it difficult to isolate this precious 'mold juice' in large quantities. It was not until 1940, just as he was contemplating retirement, that two scientists, Howard Florey and Ernst Chain, became interested in

penicillin. In time, they were able to mass-produce it for use during World War II. Fleming received many awards for his achievements.

2. Classification of Antibiotics

There are several ways of classifying antibiotics but the most common classification schemes are based on their molecular structures, mode of action and spectrum of activity (Calderon and Sabundayo, 2007). Others include route of administration (injectable, oral and topical). Antibiotics within the same structural class will generally show similar pattern of effectiveness, toxicity and allergic potential side effects. Some common classes of antibiotics based on chemical or molecular structures include Beta-lactams, Macrolides, Tetracyclines, Quinolones, Aminoglycosides, Sulphonamides, Glycopeptides and Oxazolidinones (van Hoek *et al.*, 2011; Frank and Tacconelli, 2012; Adzitey, 2015).

2.1.β-lactam

Members of this class of antibiotics contain a 3-carbon and 1-nitrogen ring that is highly reactive. They interfere with proteins essential for synthesis of bacterial cell wall, and in the process either kills or inhibits their growth. More succinctly, certain bacterial enzymes termed penicillin-binding protein (PBP) are responsible for cross linking peptide units during synthesis of peptidoglycan. Members of beta-lactam antibiotics are able to bind themselves to these PBP enzymes, and in the process, they interfere with the synthesis of peptidoglycan resulting to lysis and cell death (Heesemann, 1993). The most prominent representatives of the beta-lactam class include Penicillins, Cephalosporins, Monobactams and Carbapenems.

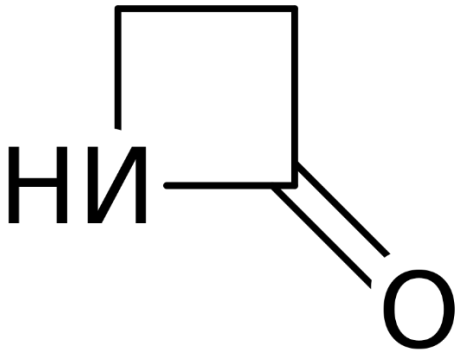


Figure 2.1.Chemical structure of a beta-lactam ring

MODE OF ACTION: Inhibit bacteria cell wall biosynthesis. (Eckburg P.B., *et al* 2019).

Adverse Effects: associated with beta-lactamase inhibitors include gastrointestinal side effects, such as diarrhea, nausea, and constipation; nervous system effects such as headaches, insomnia, and seizures; hematological effects such as impaired platelet function; allergic reactions including anaphylaxis. Beta-lactamase inhibitor use is also associated with *Candida albicans* and *Clostridioides* (*Clostridium) difficile* infections. (Holten K.B. & Onusko E.M., 2000)

2.1.1. Penicillin

The first antibiotic, penicillin, which was first discovered and reported in 1929 by Alexander Fleming was later found to be among several other antibiotic compounds called the penicillins. (McGeer *et al*., 2001). Penicillins are involved in a class of diverse group of compounds, most of which end in the suffix -cillin. They are betalactam compounds containing a nucleus of 6- animopenicillanic acid (lactam plus thiazolidine) ring and other ring side chains. As with every biological interaction systems where living systems seek to protect itself from attack, certain bacteria are able to counter the activity of antibiotics by encoding enzymes. In view of this, some antibiotics such as ampicillin, carbenicillin and amoxicillin have been developed semi-synthetically with different side-chains. These side chains confer on the antibiotics the ability to evade the degradative capacity of certain enzymes produced by certain bacterial strains as well as facilitating the movement of antibiotics across the outer membrane of such bacterial cell walls. This double-pronged capability increases their spectrum of activity against Gram-negative bacteria. In particular, some penicillins such as Augmentin are produced in combination with non-antibiotic compound that are able to inhibit the activity of bacterial penicillinase enzyme. Augmentin is actually a drug comprising amoxicillin (antibiotic) and clavulanic acid a non-antibiotic compound. Clavulanic acid is able to inhibit beta-lactamase enzyme thereby prolonging the antibacterial activity of the amoxicillin component of Augmentin even amongst penicillinase producing bacteria (Poirel *et al.,* 2005).

2.1.2. Cephalosporin

Members of this group of antibiotics are similar to penicillin in their structure and mode of action. They form part of the most commonly prescribed and administered antibiotics; more succinctly, they account for one-third of all antibiotics prescribed and administered by the National Health Scheme in the United Kingdom (Talaro and Chess, 2008). The first known member of this group of antibiotics was first isolated by Guiseppe Brotzu in 1945 from the fungus Cephalosporium acremonium. Although the drug was first isolated by Guiseppe Brotzu, it was Edward Abraham who got the credit to patent it having been able to extract the compound. Cephalosporins contain 7-aminocephalosporanic acid nucleus and side chain containing 3,6-dihydro-2 H-1,3- thiazane rings.Cephalosporins are used in the treatment of bacterial infections and diseases arising from Penicillinase-producing, Methicillin-susceptible *Staphylococci* and *Streptococci*, *Proteus mirabilis*, some *Escherichia coli*, *Klebsiella pneumonia*, *Haemophilus influenza*, *Enterobacter aerogenes* and some *Neisseria* (Pegler and Healy, 2007). They are subdivided into generations (1st -5 th) in accordance to their target organism but later versions are increasingly more effective against Gram-negative pathogens. Cephalosporins have a variety of side chains that enable them get attach to different penicillin-binding proteins (PBPs), to circumvent blood brain barrier, resist breakdown by penicillinase producing bacterial strains and ionize to facilitate entry into Gram-negative bacterial cells.

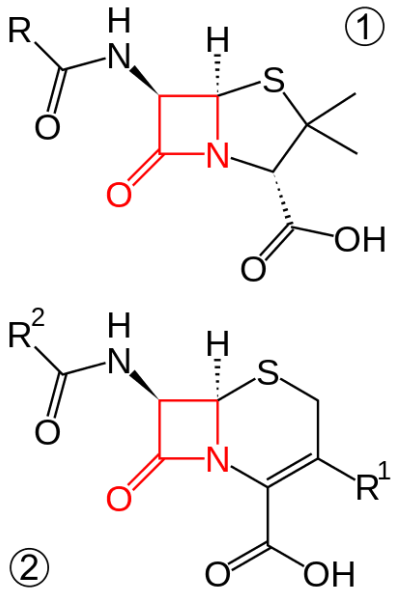


Figure 2.2. Chemical structure of betalactam structure. Core structure of penicillins (top) and cephalosporins (bottom).

2.2. Aminoglycoside

Aminoglycosides The first drug to be discovered among members of this class of antibiotics was streptomycin, first isolated in 1943 (Mahajan and Balachandran, 2012). Streptomycin has been greatly used against *Mycobacterium tuberculosis*, the causal agent of tuberculosis among humans. The aminoglycosides are compounds of usually 3-amino sugars connected by glycosidic bonds. They are obtained from soil Actimomycetes. Aminoglycoside have a broad spectrum of antibacterial activity. They are able to inhibit the protein synthesis in bacteria by binding to one of the ribosomal subunits (Peterson, 2008), and are effective against aerobic Gram-negative rods and certain Gram-positive bacteria. The oldest known aminoglycoside, as earlier inferred is Streptomycin which has been used severally in treating bubonic plague, tularemia and tuberculosis (Talaro and Chess, 2008). Notwithstanding its effectiveness against a wide array of infections, streptomycin was found to be highly toxic. This unfortunate feature of the drug necessitated the need to search for new members of aminoglycosides that would still be effective against bacteria but less toxic to humans. The search was fruitful with the discoveries of antibiotics such as Gentamicin, Neomycin, Tobramycin and Amikacin. Gentamicin is less toxic and is widely used for infections caused by Gramnegative rods (*Escherichia, Pseudomonas, Shigella and Salmonella)*. Tobramycin, in particular, is used in treating Pseudomonas infections in cystic fibrosis patients (Gilbert, 2000).

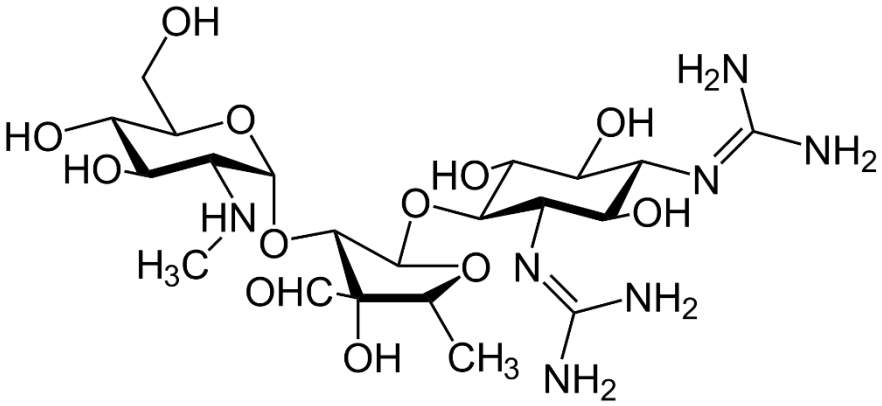


Figure 2.3. Structure of Aminoglycoside (Streptomycin)

MODE OF ACTION: Inhibit the synthesis of proteins by bacteria, leading to cell death. (Peterson, 2008).

Adverse Effects: The main noted adverse effects of aminoglycosides are ototoxicity, nephrotoxicity, and neuromuscular blockade. (Avent ML *et al* ,2011).

- ❖ Penicillins and aminoglycosides are commonly used in combination to treat a variety of infections. However, concomitant use of the extended-spectrum penicillin antimicrobials may result in inactivation of the aminoglycosides. Although the majority of interactions are reported in vitro, the potential for in vivo interactions are of concern, especially in those patients with end-stage renal failure.

2.3. Tetracycline

Tetracycline was discovered in 1945 from a soil bacterium of the genus Streptomyces by Benjamin Duggar (Sanchez *et al.,* 2004). The first member of this class was chlorotetracycline (Aureomycin). Members of this class have four (4) hydrocarbon rings and they are known by name with the suffix „–cycline". Historically, members of this class of antibiotics are grouped into different generations based on the method of synthesis. Those obtained by biosynthesis are said to be First generation. Members include Tetracycline, Chlortetecycline, Oxytetracycline and Demeclocycline. Members such as Doxycycline, Lymecycline, Meclo cycline, Methacycline, Minocycline, and Rolitetracycline are considered Second generation because they are derivatives of semi-synthesis. Those obtained from total synthesis such as Tigecycline are considered to be Third generation (Fuoco, 2012). Their target of antimicrobial activity in bacteria is the ribosome. They disrupt the addition of amino acids to polypeptide chains during protein synthesis in this bacterial organelle. Patients are advised to take tetracyclines at least two hours before or after meals for better absorption. All tetracyclines are recommended for patients above eight years because the drugs have shown to cause teeth discoloration among patients below this age can be used in treating malaria, elephantiasis, amoebic parasites and rickettisia (Sanchez *et al.,* 2004). In the past, antibiotics belonging to this class were very much the envy of numerous Clinicians owing to their wide antimicrobial spectrum but this is no longer the case because numerous bacteria are now able to resist them (Chopra and Roberts, 2001).

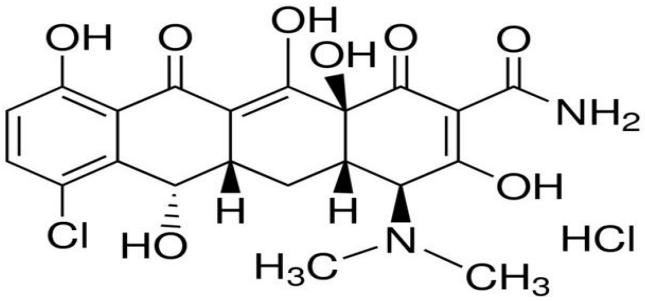


Figure 2.4. Structure of Tetracycline

MODE OF ACTION: Inhibit synthesis of proteins by bacteria, preventing growth. (Medical News Today, 2015).

Adverse Effects: nausea, vomiting, diarrhea, upset stomach, loss of appetite, white patches or sores inside your mouth or on your lips, swollen tongue, trouble swallowing, sores or swelling in your rectal or genital area, or vaginal itching or discharge. (The American Society of Health-System Pharmacists, 2016)

2.4. Macrolides

The first antibiotic belonging to this class was first discovered and isolated in 1952 by J. M. McGuire as a metabolic product of a soil inhabiting fungus *Saccharopolyspora erythraea*. This fungus was formerly known as *Streptomyces erythraeus* belonging to the genus *Saccharopolyspora* of actinomycete bacteria (Moore, 2015). Macrolides are characterized by 14-, 15-, or 16- membered macrocyclic lactose rings with unusual deoxy sugars L-cladinose and D-desosamine attached. They have a wider spectrum of antibiotic activity than Penicillins and are often administered to patients allergic to penicillin (Moore, 2015). Macrolides either kill or inhibit microorganisms by effectively inhibiting bacterial protein synthesis. They do so by binding to bacterial ribosome, and in the process, prevent the addition of amino acid to polypeptide chains during protein synthesis. Macrolides tend to build up in the body because the liver is able to recycle it into the bile. They also have the capacity to cause inflammation. As a result, clinicians usually recommend administering low doses. Although, Macrolides are generally broad spectrum, some bacterial species such as *Streptococcus pneumoniae* have resistance against the antibiotics. Example of members includes Erythromycin, Azithromycin and Clarithromycin (Hamilton-Miller, 1973).

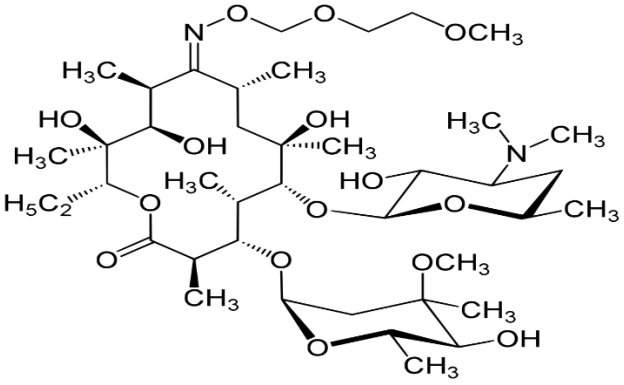


Figure 2.5. Structure of Macrolide

MODE OF ACTION: Inhibit protein synthesis by bacteria, occasionally leading to cell death. (Vázquez-Laslop N. *et al* ,2018).

Adverse Effects: Like any other antibiotic, macrolides carry a certain level of risk from typical adverse effects like nausea, vomiting, abdominal pain, and diarrhea. (Carter B.L. *et al*, 1987).

2.5. Quinolones

This class of antibiotics was first discovered as nalidixic acid by Scientists involved in search of antimalarial drugs. Nalidixic acid was discovered as an impurity during the development of quinine in the early sixties. They are able to interfere with DNA replication and transcription in bacteria. Two major groups of compounds have been developed from the basic molecule: quinolones and naphthyridones which include cinoxacin, norfloxacin, ofloxacin, ciproxacin, temafloxacin, sparfloxacin, nalidixic acid, enoxacin etc.Their structure generally consists of two rings but recent generations of quinolones possess an added ring structure which enables them to extend their spectrum of antimicrobial activity to some bacteria, particularly anaerobic bacteria that were hitherto resistant to quinolone. Since its discovery in the early 1960‟s, several modifications have been made to its parent structure and this has led to the development and synthesis of many derivatives with tested antibiotic potency. The nomenclature of members of this class of antibiotics is complex but members are often known by the suffix–oxacin, such as floxacin, ciprofloxacin and levofloxacin. Modifications in the basic structure of quinolones are reported to have improved their bioavailability and increased both their spectrum of activity and potency; enhancing their performance in the treatment of various forms of illnesses such as urinary, systemic and respiratory tract infections. Notwithstanding these notable feats, there still exist safety concerns with some members of this class of antibiotics which has led to the withdrawal of grepafloxacin, sparfloxacin, temafloxacin, trovafloxacin etc., all belonging to the class quinolones, from the market (Domagala, 1994).

Although a good deal of progress is being made in terms of in vitro studies and pharmacodynamics, knowledge of the dynamics of toxicity amongst some of this class of antibiotics is yet inconclusive.

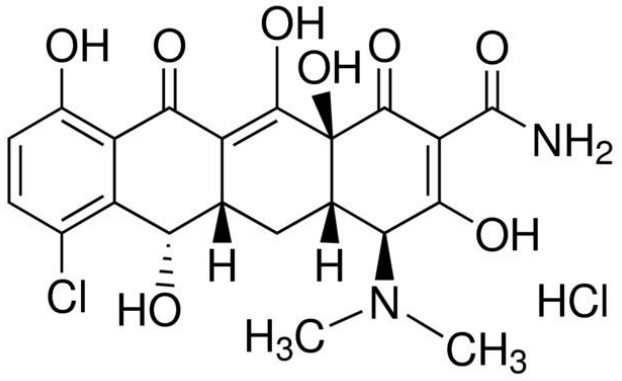


Figure 2.6. Structure of Quinolone

MODE OF ACTION: Interfere with bacteria DNA replication and transcription. (Mandell G.L. *et al*, 2000).

Adverse Effects: Quinolones have few adverse effects, most notably nausea, headache, dizziness, and confusion. (S.t. Louis, 2000).

2.6. Sulphonamides

Sulphonamides are reportedly, the first group of antibiotics used in therapeutic medicine, and they still play very important role in medicine and veterinary practice (Eyssen *et al.*, 1971). Sulphonamides inhibit both Gram-positive and Gram-negative bacteria such as *Nocardia*, *E. coli, Klebsiella, Salmonella, Shigella* and *Enterobacter*, *Chlamydia trachomatis* and some Protozoa, and are widely used in the treatment of various infections including tonsillitis, septicemia, meningococcal meningitis, bacillary dysentery and some urinary tract infections (Eyssen et al., 1971). Studies have shown that Sulphonamides are also able to impede cancerous cell agents (Stawinski *et al.*, 2013; Xu *et al.*, 2014). The original antibacterial sulphonamide (also spelt sulfonamide by some Workers), are synthetic antimicrobial agents that contain the sulphonamide group (Henry, 1943). Sulphonamides are generally thought to be bacteriostatic rather than bactericidal. However, Henry (1943) in his thorough early work opined that sulphonamides may become bactericidal if their concentration is sufficiently high or if the presence of any sulfonamide concentration is accompanied by other environmental conditions

unfavourable to bacteria. Such unfavourable conditions would include poor cultural conditions, adverse temperature, antibodies, toxic proteolytic product etc. Although sulphonamides are adjudged good and effective in treating various diseases and infections, they are recommended and administered with caution because of their toxicity and side effects, some of which include urinary tract disorders, haemolytic anaemia, porphyria, and hypersensitivity reactions (Choquet-Kastylevsky *et al*., 2002;Slatore and Tilles, 2004).

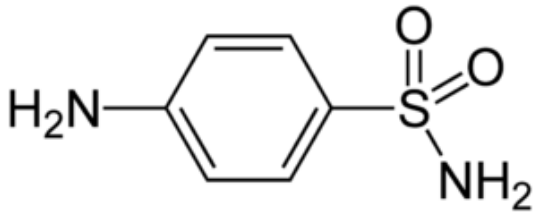


Figure 2.7. Structure of Sulphonamides

MODE OF ACTION: Antibiotics are chemotherapeutic agents used to inhibit or kill bacteria. Sulphonamides are competitive antagonists and structural analogues of *p*-aminobenzoic acid (PABA) in the synthesis of folic acid which is essential for the further production of DNA in the bacteria (Zessel *et al.*, 2014).

Adverse Effects: Sulfonamides cause side effects in 4-6% of the treated patients from general population and even 50-60% of patients with HIV infection(R. S. Gruchalla *et al* ,2000;C. C. Brackett, *et al* ,2004).Hypersensitivity is the most common adverse reaction to sulfonamides and it is often referred to as 'sulfa allergy'.

In some patients, urinary tract disorder occurs as a result of metabolite precipitation (acetylated sulfonamides), especially in the case of acidic urine. (V. M. Varagić, *et al*, 2009).

3. Mechanisms of Action

3.1. Basic Anatomy of Bacterial Cell

The Gram-positive bacteria consists of cytoplasmic membrane surrounded by a tough and rigid mesh called cell wall. In contrast, Gram-negative bacteria consist of thin cell wall that is surrounded by second lipid membrane called outer membrane (OM). The space between the OM and cytoplasmic membrane is referred as periplasm. The OM is an additional protective layer in Gram-negative bacteria and prevents many substances from entering into the bacterium. However, this membrane contains channels called porins, which allow the entry of various molecules such as drugs (Hauser A.R., 2015). The cell wall is a tough layer that gives bacterium a characteristic shape and prevents it from osmotic and mechanical stresses. The cytoplasmic membrane prevents ions from flowing into or out of the cell and maintains the cytoplasmic and bacterial components in a defined space.

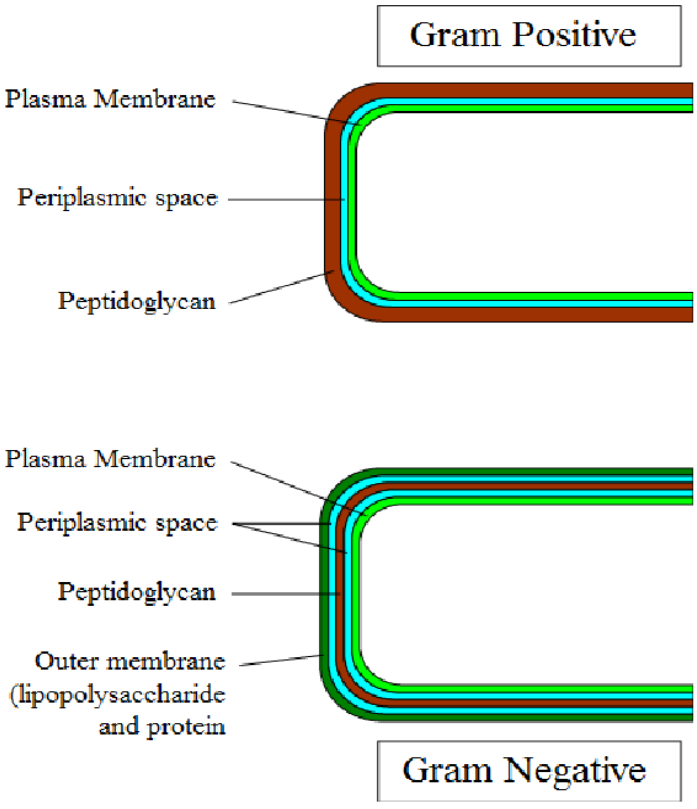


Figure 3.1. Structure of bacterial cell envelope

3.2. How do antibiotics work?

Pathogenic microorganisms can infect tissues of human by destroying cellular functions. Microorganisms themselves or their toxins can damage host cells. Microbial infections are treated with antimicrobials by either inhibiting the microbial growth or killing the microorganism. Antibiotics are widely being used not only in the treatment of acute and chronic infections, but also in the prophylactic treatment (Wiley & Sons, 2011). Targets of antimicrobials are cell membrane, cell wall, protein synthesis, nucleic acid synthesis, and biological metabolic compound synthesis.

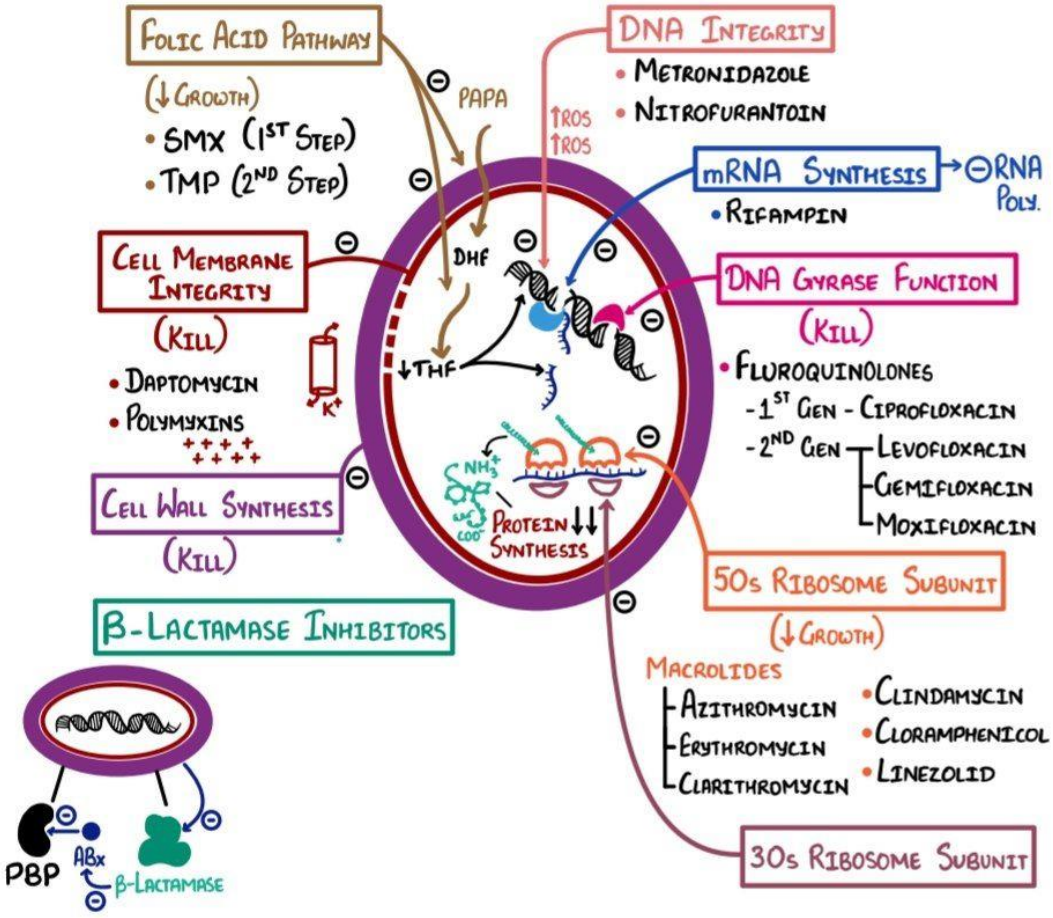


Figure 3.2. Mechanism of Action of Antibiotics

3.3. Classification of Antibiotics on the Basis of Mechanism of Action

- The mechanism of action of antibiotics is best categorized **based on the structure of the bacteria.**
- Either a bactericidal or bacteriostatic
  - **Bactericidal** – Kills the bacteria.
  - **Bacteriostatic** – Inhibits the growth of the bacteria.

3.3.1. Antibiotics targeting cell wall

Bacterial cells are surrounded by a cell wall made of peptidoglycan, which consists of long sugar polymers. The peptidoglycan undergoes cross-linking of the glycan strands by the action of transglycosidases, and the peptide chains extend from the sugars in the polymers and form cross links, one peptide to another (Kahne D *et al*, 2005). The D-alanyl-alanine portion of peptide chain is cross linked by glycine residues in the presence of penicillin binding proteins (PBPs).This cross-linking strengthens the cell wall. β-lactams and the glycopeptides inhibit cell wall synthesis.

Beta-lactam antibiotics

The primary targets of the β-lactam agents are the PBPs. It has been hypothesized that the β-lactam ring mimics the D-alanyl D-alanine portion of peptide chain that is normally bound by PBP. The PBP interacts with β-lactam ring and are not available for the synthesis of new peptidoglycan. The disruption of peptidoglycan layer leads to the lysis of bacterium (Džidic S. *et al*, 2008).

Glycopeptides

The glycopeptides binds to D-alanyl D-alanine portion of peptide side chain of the precursor peptidoglycan subunit. The large drug molecule vancomycin prevents binding of this D-alanyl subunit with the PBP, and hence inhibits cell wall synthesis (Grundmann H. *et al*, 2006; Džidic S. *et al*, 2008).

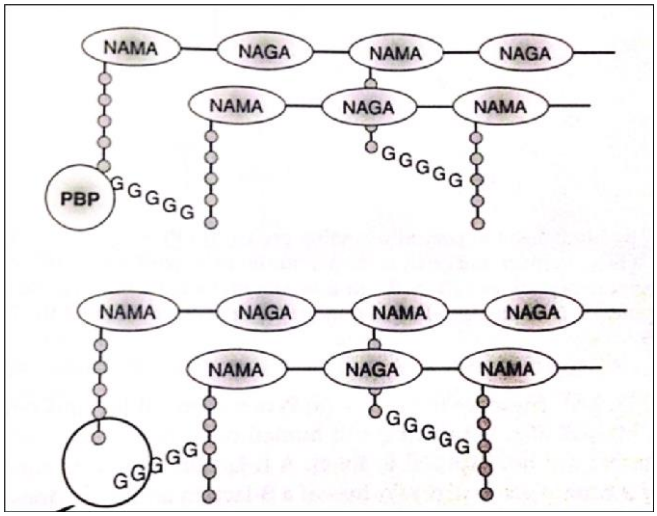


Figure 3.3. Mechanism of action of β-lactam antibiotics

3.3.2. Inhibitors of protein biosynthesis

First the information in bacterial DNA is used to synthesize an RNA molecule referred to as messenger RNA (m-RNA) a process known as transcription .Then, the macromolecular structure called ribosome synthesizes proteins present in m-RNA, a process called translation. Protein biosynthesis is catalyzed by ribosomes and cytoplasmic factors. The bacterial 70S ribosome is composed of two ribonucleoprotein subunits, the 30S and 50S subunits (Yoneyama H. & Katsumata R., 2006). Antimicrobials inhibit protein biosynthesis by targeting the 30S or 50S subunit of the bacterial ribosome (Johnston N.J. *et al*, 2002).

Inhibitors of 30S subunit

Aminoglycosides

The aminoglycosides (AG's) are positively-charged molecules which attach to the OM which is negatively charged leading to formation of large pores, and thus allow antibiotic penetration inside the bacterium. The main target of action is bacterial ribosome; to enter, there it must pass through cytoplasmic membrane requiring energy dependent active bacterial transport mechanism, which requires oxygen and an active proton motive force. For these reasons, AG work in aerobic conditions and have poor activity against anaerobic bacteria. These AG have synergism with those antibiotics, which inhibit cell wall synthesis (such as β-lactam and glycopeptides) as it allows greater penetration of AG within the cell and at low dosages. AG's interact with the 16S r-RNA of the 30S subunit near the A site through hydrogen bonds. They cause misreading and premature termination of translation of mRNA.

Tetracyclines

Tetracyclines, such as tetracycline, chlortetracycline, doxycycline, or minocycline, act upon the conserved sequences of the 16S r-RNA of the 30S ribosomal subunit to prevent binding of t-RNA to the A site (Yoneyama H. & Katsumata R., 2006).

Inhibitors of 50S subunit

Chloramphenicol

It interacts with the conserved sequences of the peptidyl transferase cavity of the 23S r-RNA of the 50S subunit. Hence, it inhibits the protein synthesis by preventing binding of t-RNA to the A site of the ribosome (Yoneyama H. & Katsumata R., 2006).

Macrolides

These affect the early stage of protein synthesis, namely translocation, by targeting the conserved sequences of the peptidyl transferase center of the 23S r-RNA of the 50S ribosomal subunit (Yoneyama H. & Katsumata R., 2006).

Oxazolidinones

Linezolid is a recently approved member of novel class of antibiotic of this group which is completely synthetic. Oxazolidinones interfere with protein synthesis at several stages:

(i) Inhibit protein synthesis by binding to 23Sr RNA of the 50S subunit and
(ii) Suppress 70S inhibition and interact with peptidyl-t-RNA (Lambert P.A., 2005).

3.3.3. Inhibitors of DNA replication

Quinilones

The fluoroquinolones (FQ) inhibit the enzyme bacterial DNA gyrase, which nicks the double-stranded DNA, introduces negative supercoils and then reseals the nicked ends. This is necessary to prevent excessive positive supercoiling of the strands when they separate to permit replication or transcription. The DNA gyrase consists of two A subunits and two B subunits. A subunit carries out the nicking of DNA, B subunit introduces negative supercoils, and then A subunit reseal the strands. The FQ's bind to A subunit with high affinity and interfere with its strand cutting and resealing function. In Gram-positive bacteria, the major target of action is topoisomerase IV which nicks and separate's daughter DNA strand after DNA replication. Greater affinity for this enzyme may confer higher potency against Gram-positive bacteria. In place of DNA gyrase or topoisomerase IV, mammalian cells possess topoisomerase II, which has very low affinity for FQ-hence low toxicity to cells (Higgins P.G. *et al*, 2003; Yoneyama H. & Katsumata R., 2006).

3.3.4. Folic acid metabolism inhibitors

Sulfonamides and trimethoprim

Each of these drugs inhibits distinct steps in folic acid metabolism. A combination of sulpha drugs and trimethoprim acting at distinct steps on the same biosynthetic pathway shows synergy and a reduced mutation rate for resistance. Sulfonamides inhibit dihydropteroate synthase in a competitive manner with higher affinity for the enzyme than the natural substrate, p-amino benzoic acid. Agents such as trimethoprim act at a later stage of folic acid synthesis and inhibit the enzyme dihydrofolate reductase (Yoneyama H. & Katsumata R., 2006).

4. Mechanisms of Resistance to Antibiotic

Resistance is the ability of a bacteria against the antagonizing effect of an antibacterial agent upon reproduction prevention or bactericidal. The development of resistance to antibiotics in bacteria often develop as a result of unnecessary and inappropriate use of antibiotics. Through the intense use of antibiotics, resistant microorganisms have emerged over the years, and problems were started to be experienced for the treatment of these infections emerged with these resistant microorganisms. Today, on the one hand trying to develop new drugs, on the other hand, there are difficulties in treatment as a result of development of resistance to these drugs rapidly. The development of resistance to antibiotics is a major public health problem in all over the world (Yüce A., 2001).

4.1. The main four types of resistance to antibiotics develops:

1. Natural (Intrinsic) resistance.

2. Acquired resistance.

3. Cross-resistance.

4. Multi-drug resistance and pan-resistance.

4.1.1. Natural (Intrinsic, Structural) resistance: This kind of resistance is caused by the structural characteristics of bacteria and it is not associated with the use of antibiotics. It has no hereditary property. It develops as result of the natural resistance, or the microorganisms not including the structure of the target antibiotic, or antibiotics not reaching to its target due to its characteristics. For example, Gram-negative bacteria Vancomycin does not pass in the outer membrane so Gram-

negative bacteria is naturally resistant to Vancomycin. Similarly, L-form shape of bacteria which are wall-less forms of the bacteria, and the bacteria such as cell wall-less cell Mycoplasma and Ureaplasma are naturally resistant to beta-lactam antibiotics that inhibit the cell wall synthesis. (Yüce A., 2001; Nikaido H., 2009)

4.1.2. Acquired resistance: As result of the changes in the genetic characteristics of bacteria, an acquired resistance occurs due to its not being affected from the antibiotics it has been responsive before. This kind of resistance occurs due to mainly structures of chromosome or extra chromosomal (plasmid, transposon, etc.).

4.1.2.1. Chromosomal resistance arise from mutations in developing in spontaneous bacterial chromosome (spontaneous). Such mutations may occur according to some physical (ultraviolet, etc.) and chemical factors. This can be a result of structural changes in bacterial cells. The result may be reduced permeability of bacterial drug or changes of the target of the drug may be in the cell. Streptomycin, aminoglycosides, erythromycin, lincomycin can develop resistance against these types. (Yüce A., 2001).

4.1.2.2. Extra chromosomal resistance depends extra chromosomal genetic elements that can be transferred in various ways like plasmids, transposons and integro. Plasmids are extra chromosomal DNA fragments that can replicate independently from chromosome. Plasmid genes are usually responsible for the generation of enzymes which inactive antibiotics. Resistance genes and plasmids carrying the genetic material from a bacterium in three ways those are transduction, transformation, conjugation, and transposition mechanism. (Yüce A., 2001).

4.1.3. Cross resistance: Some microorganisms which are resistant to a certain drug, that acts with the same or similar mechanism and also resistant to other drugs. This condition is usually observed in antibiotics whose structures are similar: such as resistance between erythromycin, neomycin-kanamycin or resistance between cephalosporins and penicillins. However, sometimes it can also be seen in a completely unrelated drug groups. There is an example of cross-resistance between erythromycin-lincomycin. This may be chromosomal or extra chromosomal origin (Jawetz E., 1995; Mayer K.H., 1995).

4.1.4. Multi-drug resistance and pan-resistance: Multidrug-resistant organisms are usually bacteria that have become resistant to the antibiotics used to treat them. This means that a particular drug is no longer able to kill or control the bacteria. Inapropriate use of antibiotics for therapy resulted in the selection of pathogenic bacteria resistant to multiple drugs. Multidrug resistance in bacteria can be occured by one of two mechanisms. First, these bacteria may accumulate multiple genes, each coding for resistance to a single drug. This type of resistance occurs typically on resistance (R) plasmids. Second type of resistance, namely multidrug resistance may also occur by the increased expression of genes that code for multidrug efflux pumps, enzymatic inactivation, changes in the structure of the target etc. If the bacterial strains resistant to three or more classes of antimicrobials, it is considered as multi-drug resistant. If the strains, resistant to all but one or two antibiotic gruops, they are considered as extensively-drug-resistant, if the strains resistant to all available antibiotic, they are classified as pan-drug-resistant. For example, multidrug resistance (MDR) Acinetobacter species (spp.) can be defined

as the isolate resistant to at least three classes of antimicrobial agents (namely, all penicillins and cephalosporins (including inhibitor combinations), fluroquinolones, and aminoglycosides). Extensive drug resistant (XDR) Acinetobacter spp.' shall be the Acinetobacter spp. isolate that is resistant to the three classes of antimicrobials described above (MDR) and shall also be resistant to carbapenems. Pandrug resistant or pan-resistant (PDR) Acinetobacter spp. shall be the XDR Acinetobacter spp. that is resistant to polymyxins (colistin) and tigecycline.(Eliopoulos G.M., *et al*.,2008 ;Nikaido H., 2009;Vikas Manchanda *et al.*, 2010).

4.2. 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.

Intrinsic resistance may make use of limiting uptake, drug inactivation, and drug efflux; acquired resistance mechanisms used may be drug target modification, drug inactivation, and drug efflux. Because of differences in structure, etc., there is variation in the types of mechanisms used by gram negative bacteria versus gram positive bacteria. Gram negative bacteria make use of all four main mechanisms, whereas gram positive bacteria less commonly use limiting the uptake of a drug (don't have an LPS outer membrane), and don't have the capacity for certain types

of drug efflux mechanisms (refer to the drug efflux pumps later in this manuscript) (Chancey S.T. *et al*, 2012; Mahon C.R., *et al*, 2014).

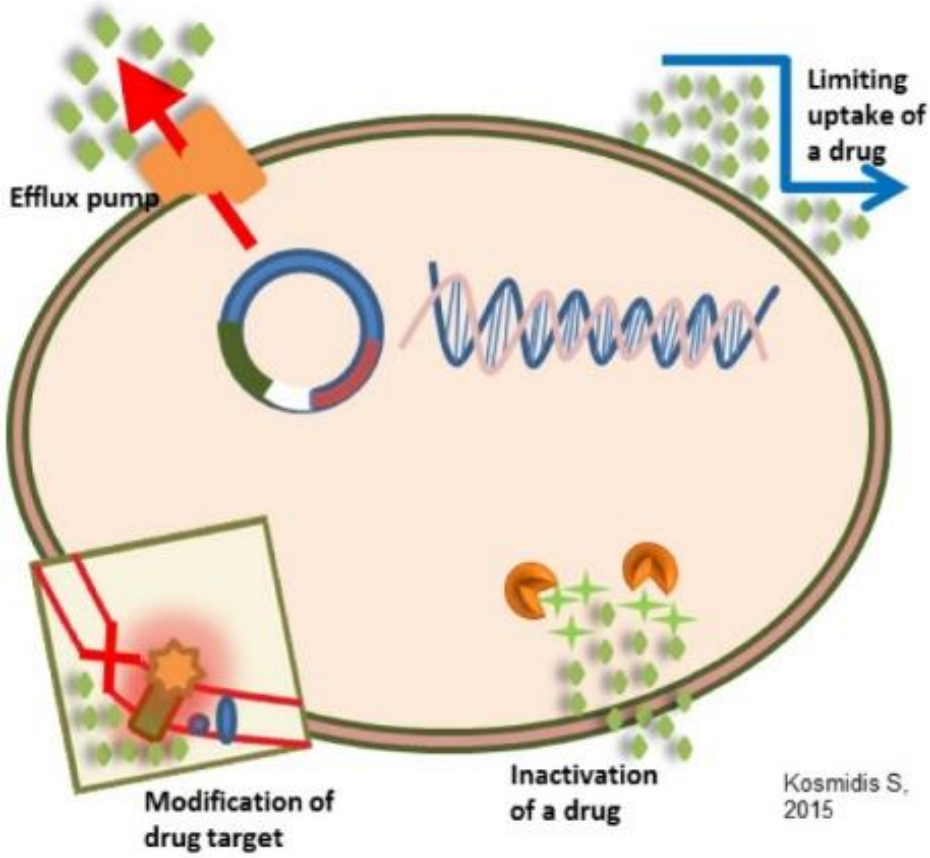


Figure 4.1. General antimicrobial resistance mechanisms.

4.2.1. Limiting drug uptake

As already mentioned, there is a natural difference in the ability of bacteria to limit the uptake of antimicrobial agents. 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 (Blair J.M. *et al*, 2014). 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 (Lambert P.A., 2002; Kumar A. & Schweizer H.P., 2005). 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 (Bébéar C.M. and Pereyre S.,2005).Gram positive bacteria do not possess an outer membrane, and restricting drug access is not as prevalent. In the enterococci, the fact that polar molecules have difficulty penetrating the cell wall gives intrinsic resistance to aminoglycosides. Another gram positive bacteria, *Staphylococcus aureus*, recently has developed resistance to vancomycin. Of the two mechanisms that *S. aureus* uses against vancomycin, a yet unexplained mechanism allows the bacteria to produce a thickened cell wall which makes it difficult for the drug to enter the cell, and provides an intermediate resistance to vancomycin. These strains are designated as VISA strains (Lambert P.A., 2002; Miller W.R., *et al*, 2014). In those bacteria with large outer membranes, substances often enter the cell through porin channels. The porin channels in gram negative bacteria generally allow access to hydrophilic molecules (Blair J.M. *et al*, 2014). There are two main ways in which porin changes can limit drug uptake: a decrease in the number of porins present, and mutations that change the selectivity of the porin channel (Kumar A. & Schweizer H.P., 2005). Members of the *Enterobacteriaceae* are known to become resistant due to reducing the number of porins (and sometime stopping production entirely of certain porins). As a group, these bacteria reduce porin number as a mechanism for resistance to carbapenems (Cornaglia G. *et al*, 1996). Mutations that cause changes within the porin channel have been seen in *E. aerogenes* which then become resistant to imipenem and certain cephalosporins, and in *Neisseria gonorrhoeae* which then become resistant to β-lactams and tetracycline (Thiolas A. *et al*, 2004).

Another widely seen phenomenon in bacterial colonization is the formation of a biofilm by a bacterial community. These biofilms may contain a predominant organism (such as by *Pseudomonas aeruginosa* in the lung), or may consist of a wide variety of organisms, as seen in the biofilm community of normal flora in the gut. For pathogenic organisms, formation of a biofilm protects the bacteria from attack by the host immune system, plus provides protection from antimicrobial agents. 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. In addition the bacterial cells in the biofilm tend to be sessile (slow metabolism rate, slow cell division), so antimicrobials that target growing, dividing bacterial cells have little effect. An important observation about biofilms is that it is likely that horizontal transfer of genes is facilitated by the proximity of the bacterial cells. That means that sharing of antimicrobial resistance genes is potentially easier for these bacterial communities (Mah T.F., 2012; Soto S.M., 2013; Van Acker H. *et al*, 2014).

4.2.2. Modification of drug targets

There are multiple components in the bacterial cell that may be targets of antimicrobial agents; and there are just as many targets that may be modified by the bacteria to enable resistance to those drugs. 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). PBPs are transpeptidases involved in the construction of peptidoglycan in the cell wall. A change in the number (increase in PBPs that have a decrease in drug binding ability, or decrease in PBPs with normal drug binding) of PBPs impacts the amount of drug that can bind to that target. A change in structure (e.g. PBP2a in *S. aureus* by

acquisition of the *mecA* gene) may decrease the ability of the drug to bind, or totally inhibit drug binding (Reygaert W.C., 2009; Beceiro A. *et al*, 2013).

The glycopeptides (e.g. vancomycin) also work by inhibiting cell wall synthesis, and lipopeptides (e.g. daptomycin) work by depolarizing the cell membrane. Gram negative bacteria (thick LPS layer) have intrinsic resistance to these drugs (Randall C.P. *et al*, 2013). Resistance to vancomycin has become a major issue in the enterococci (VRE—vancomycin-resistant enterococci) and in *Staphylococcus aureus* (MRSA). Resistance is mediated through acquisition of *van* genes which results in changes in the structure of peptidoglycan precursors that cause a decrease in the binding ability of vancomycin (Cox G. & Wright G.D., 2013; Beceiro A. *et al*, 2013). Daptomycin requires the presence of calcium for binding. Mutations in genes (e.g. *mprF*) change the charge of the cell membrane surface to positive, inhibiting the binding of calcium, and therefore, daptomycin (Yang S.J. *et al*, 2009; Mishra N.N. *et al*, 2014; Stefani S. *et al*, 2015).

Resistance to drugs that target the ribosomal subunits may occur via ribosomal mutation (aminoglycosides, oxazolidinones), ribosomal subunit methylation (aminoglycosides, macrolides—gram positive bacteria, oxazolidinones, streptogramins) most commonly involving *erm* genes, or ribosomal protection (tetracyclines). These mechanisms interfere with the ability of the drug to bind to the ribosome. The level of drug interference varies greatly among these mechanisms (Roberts M.C., 2003; Roberts M.C., 2004; Kumar S. *et al*, 2013).For drugs that target nucleic acid synthesis (fluoroquinolones), resistance is via modifications in DNA gyrase (gram negative bacteria—e.g. *gyrA*) or topoisomerase IV (gram positive bacteria—e.g. *grlA*). These mutations cause changes in the structure of gyrase and topoisomerase which decrease or eliminate the ability of the drug to bind to these components (Hawkey P.M., 2003; Redgrave L.S.*et al*, 2014).

For the drugs that inhibit metabolic pathways, resistance is via mutations in enzymes (DHPS—dihydropteroate synthase, DHFR—dihydrofolate reductase) involved in the folate biosynthesis pathway and/or overproduction of resistant DHPS and DHFR enzymes (sulfonamides—DHPS, trimethoprim—DHFR). The sulfonamides and trimethoprim bind to their respective enzymes due to their being structural analogs of the natural substrates (sulfonamides—*p*-amino-benzoic acid, trimethoprim—dihydrofolate). The action of these drugs is through competitive inhibition by binding in the active site of the enzymes. Mutations in these enzymes are most often located in or near the active site, and resulting structural changes in the enzyme interfere with drug binding while still allowing the natural substrate to bind (Vedantam G. *et al*, 1998).

4.2.3. Drug inactivation

There are two main ways in which bacteria inactivate drugs; by actual 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. Another drug that can be inactivated by hydrolyzation is tetracycline, via the *tetX* gene (Kumar S. *et al*, 2013; Blair J.M. *et al* ,2015). Drug inactivation by transfer of a chemical group to the drug most commonly uses transfer of acetyl, phosphoryl, and adenyl groups. There are a large number of transferases that have been identified. Acetylation is the most diversely used mechanism, and is known to be used against the aminoglycosides, chloramphenicol, the streptogramins, and the fluoroquinolones. Phosphorylation and adenylation are known to be used primarily against the aminoglycosides (Robicsek A, *et al*, 2006; Ramirez M.S. & Tolmasky M.E., 2010; Blair J.M. *et al*, 2015).

4.2.4.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). The resistance capability of many of these pumps is influenced by what carbon source is available (Villagra N.A. *et al*, 2012; Blair J.M. *et al*, 2014). Most bacteria possess many different types of efflux pumps. There are five main families of efflux pumps in bacteria classified based on structure and energy source: the ATP-binding cassette (ABC) family, the multidrug and toxic compound extrusion (MATE) family, the small multidrug resistance (SMR) family, the major facilitator superfamily (MFS), and the resistance-nodulation-cell division (RND) family. Most of these efflux pump families are single-component pumps which transport substrates across the cytoplasmic membrane. The RND family are multi-component pumps (found almost exclusively in gram negative bacteria) that function in association with a periplasmic membrane fusion protein (MFP) and an outer membrane protein (OMP-porin) to efflux substrate across the entire cell envelope (Kumar A. & Schweizer H.P., 2005; Piddock L.J. ,2006; Poole K. ,2007; Villagra N.A. *et al*, 2012). There are instances where other efflux family members act with other cellular components as multicomponent pumps in gram negative bacteria. One member of the ABC family, MacB, works as a tripartite pump (MacAB-TolC) to extrude macrolide drugs. A member of the MFS, EmrB, works as a tripartite pump (EmrAB-TolC) to extrude nalidixic acid in *E. coli* (Tanabe M. *et al*, 2009; Jo I. *et al*, 2017).

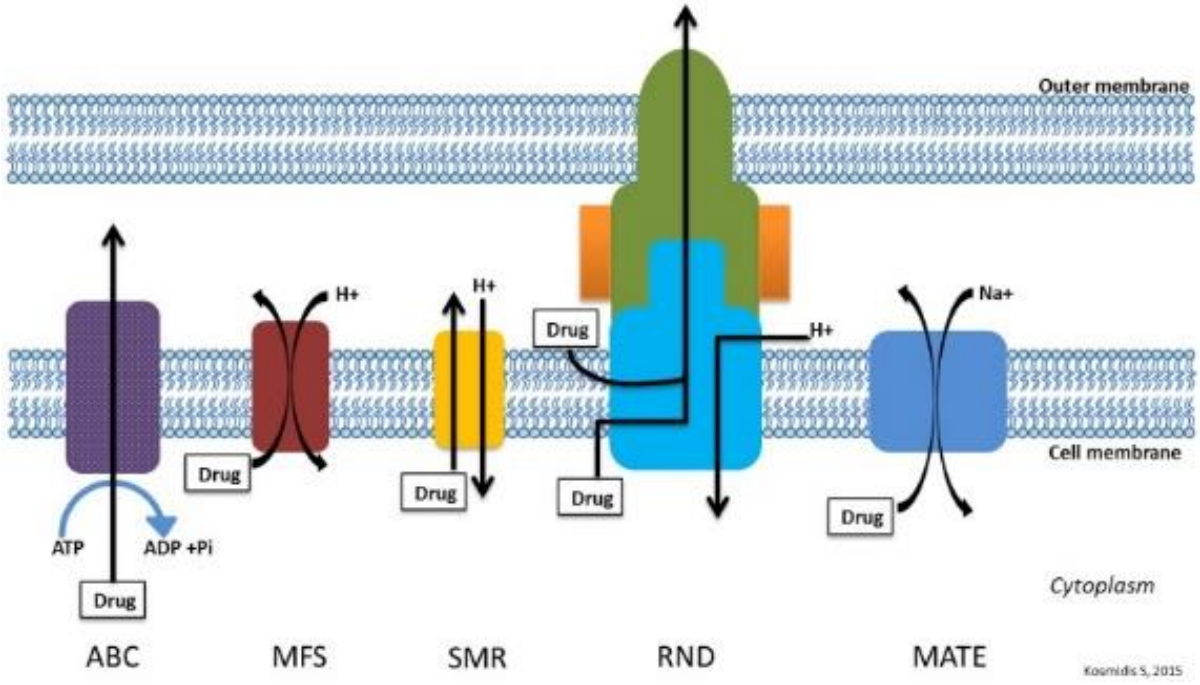


Figure 4.2. General structure of main efflux pump families

Efflux pumps found in gram positive bacteria may confer intrinsic resistance because of being encoded on the chromosome. These pumps include members of the MATE and MFS families and efflux fluoroquinolones. There are also gram positive efflux pumps known to be carried on plasmids. Currently, the characterized pumps in gram positive bacteria are from the MFS family (Costa S.S. *et al*, 2013; Kourtesi C. *et al*, 2013). Efflux pumps found in gram negative bacteria are widely distributed and may come from all five of the families, with the most clinically significant pumps belonging to the RND family (Kourtesi C. *et al*, 2013; Blair J.M. *et al*, 2014).

5. MDR, XDR and PDR

Emergence of resistance to multiple antimicrobial agents in pathogenic bacteria has become a significant public health threat as there are increasingly fewer, or even no effective antimicrobial agents available for infections caused by these bacteria. Gram-positive and gram-negative bacteria are both affected by the emergence and rise of antimicrobial resistance. As this problem continues to grow, harmonized definitions with which to describe and classify bacteria that are resistant to multiple antimicrobial agents are needed, so that epidemiological surveillance data can be reliably collected and compared across healthcare settings and countries. In the strictest sense, multidrug-resistant organisms (MDROs) are labeled as such because of their resistance to more than one antimicrobial agent. Infections with MDROs can lead to inadequate or delayed antimicrobial therapy, and are associated with poorer patient outcomes (Ibrahim E.H. *et al*, 2000; Cosgrove S.E. *et al*, 2003; Anderson D.J. *et al*, 2006; Roberts R.R. *et al*, 2009).

Infections caused by drug-resistant gram-negative bacteria (GNB), particularly the hospital-acquired antibiotic-resistant infections pose a significant threat to global public health.(Magiorakos A.P. *et al*,2012; Exner M. *et al*,2015) Organisms expressing in vitro resistance to three or more antimicrobial classes are referred to as multidrug-resistant organisms.(Magiorakos A.P. *et al*,2012) According to the Centers for Disease Control and Prevention (CDC), more than 70% of the bacteria causing hospital-acquired infections are resistant to at least one of the antimicrobial agents that are commonly used to treat them(Kang C.I. *et al*,2005). Enterobacteriaceae *Pseudomonas aeruginosa* and *Acinetobacter baumannii* are the most common hospital acquired drug-resistant GNBs. (Lockhart S.R. *et al*, 2007).

There are three types of antimicrobial resistance exhibiting microorganisms:

- Multidrug-resistant (MDR).
- Extensively drug-resistant (XDR).
- Pan drug-resistant (PDR).

The definitions of MDR, XDR, and PDR strains were based on the standardized international terminology proposed by CDC and the European Centre for Disease Prevention and Control (ECDC) standardized international terminology. (Magiorakos A.P. *et al*, 2012).

5.1. MDR was defined as acquired non-susceptibility to at least one agent in three or more antimicrobial categories.

5.2. XDR was defined as non-susceptibility to at least one agent in all but two or fewer antimicrobial categories (i.e. bacterial isolates remain susceptible to only one or two categories). And

5.3. PDR was defined as non-susceptibility to all agents in all antimicrobial categories (Magiorakos A.P. *et al*, 2012).

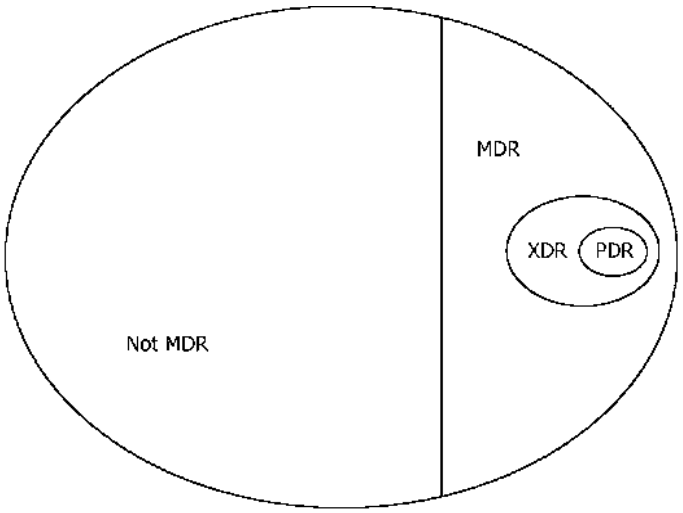


Figure 5. Diagram showing the relationship of MDR, XDR and PDR to each other

Initially, the term XDR was used to describe extensively drug-resistant Mycobacterium tuberculosis (XDR MTB) and was defined as 'resistance to the first-line agent's isoniazid and rifampicin, to a fluoroquinolone and to at least one of the three-second-line parenteral drugs (i.e. amikacin, kanamycin or capreomycin).

Two sets of criteria have mainly been used to characterize bacteria as XDR. The first is based on the number of antimicrobials or classes or subclasses to which a bacterium is resistant, and the second one whether they are 'resistant to one or more key antimicrobial agents (Siegel J.D. *et al*, 2007;Cohen A.L. *et al*, 2008; Hidron A.I. *et al*, 2008). PDR 'Pan' means 'all', pan drug resistant (PDR) means 'resistant to all antimicrobial agents'. It means that, to characterize a bacteria as PDR, it must be tested and found to be resistant to all approved and useful antimicrobials. Examples of current definitions are: 'resistant to almost all commercially available antimicrobials', 'resistant to all antimicrobials routinely tested 'and 'resistant to all antibiotic classes available for empirical treatment' (Kuo L.C. *et al*, 2003;Kuo L.C. *et al*, 2004; Falagas M.E. *et al*, 2006).

How to treat these bacteria?

Some alternative drugs are available to treat MDR bacteria and very few options available to treat XDR bacteria. But almost no option is available to treat PDR bacteria. To treat PDR, either new effective antibiotics are to be discovered, alternatively best possible combinations of two or more antibiotics are to be tried. Against infections with *Pseudomonas aeruginosa* or *Acinetobacter baumannii* isolates that are resistant to all antibiotics except the polymyxins, several novel antibiotic combinations demonstrate increased activity in vitro compared with that of any single agent (Farzana A., 2013; Rahal J.J., 2016; Uddin B.M.M., 2016).

When two antibiotics are used in combination, the best and most desirable effect is synergism and the worst one is antagonism, summation and indifference are in between (Brooks G.F. *et al*, 2013). In vitro synergistic effects have been observed against *Pseudomonas aeruginosa* (Farzana A., 2013), and in vitro and in vivo in rat model synergistic effects have been observed against *Acinetobacter baumannii* using combinations of amikacin with carbapenems, and carbapenem with ceftazidyme (Uddin B.M.M., 2016).

6. Technique and method used to determine the antibiotic resistance

Antimicrobial products kill or slow the spread of microorganisms. Microorganisms include bacteria, viruses, protozoans, and fungi such as mold and mildew.

Antimicrobial Resistance (AMR) has become one of the dominant health challenges of our times. Antibiotic resistance occurs as a natural evolutionary process in bacteria, but can be accelerated by a number of factors (Collignon P. C. *et al*, 2016; McAdams D. *et al*, 2019). More specifically, the excessive and inadequate use of antibiotics in both humans and animals leads to the wide spread of resistant bacteria and their antimicrobial resistant genes (ARGs) (Ferri M. *et al*,2017;Leonard H. *et al*,2018;Hashempour-Baltork F. *et al*,2019). AMR has severe adverse effects on humans, healthcare systems, farm animals, agriculture, environmental health, and, consequently, on national economies (Friedman N.D. *et al*, 2016).AMR is a challenging threat undermining key features of current medical care at enormous costs in terms of patient mortality and morbidity, but also in terms of patient treatment expenses (O'Neill, J. 2016; Roope L. S. *et al*, 2019). Modern, mainstream antibiotic therapeutic strategies are responsible for their own regression by actively

selecting for resistant strains, compelling the need for supporting the continuous discovery of new antibiotics in order to remain ahead of the AMR challenge (Bell, G. & MacLean C. ,2018). Therefore, it is urgent to prolong the lifespan of current antibiotics while research and development of new-generation antibiotics takes its course. In addition, it is important to implement efficient control measures for antibiotic use in order to slow down the need for continuous discovery of new antibiotics (McAdams D. *et al*, 2019).

6.1. Molecular techniques

Molecular techniques enable the detection of genetic material, both deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). Polymerase chain reaction (PCR) is the molecular technique that has acquired the greatest diagnostic value, since it not only allows the infectious agent to be accurately identified, but is also the leading method to characterize its resistance and virulence genotypes. Conventional PCR requires approximately 12 h to perform and consists of 3 steps. The first step consists of extraction of genetic material. The second step, performed in a thermo cycler, consists of DNA amplification. The thermo cycler reaches the optimal temperatures required for each of the 3 steps comprising an amplification cycle (denaturation of the DNA to be used as a mould, ringing of synthetic primers and extension catalyzed by the polymerase DNA of the primers) to take place. Amplification is repeated a certain number of times, generally 25–35. Each time, the number of product molecules (amplicons) is duplicated. Thus a high number of amplicons is synthesized, which allows very small initial amounts of DNA to be detected(Rosselló *et al*, 2016).

6.2. Microarrays

This method is based on using an image analysis to detect hybridization of a target molecule to a specific probe immobilized on a solid base. Microarrays detect a large number of resistance genes in a single assay given that these probes, which are normally oligonucleotides, are attached very close to one another. Several microarrays have been marketed, such as the Check-MDR CT102, the Check-MD CT103 and the Check-MDR CT103 XL (Check points Health BV). These microarrays detect a large number of genes that encode different beta-lactamases (ESBLs, AmpCs and carbapenemases) based on colonies grown on isolation plates. These 3 microarrays require a first step of a PCR with a pair of universal primers marked with biotin. Next, the amplicons are classified through hybridisation with the oligonucleotide probes. Finally, the manufacturer's software program detects hybridisation using the biotin marker, automatically translates the data and expresses the results in the form of the presence or absence of a gene. These microarrays yield results in 8 h and have a sensitivity and a specificity of practically 100% (Stuart J.C *et al*, 2012; Cuzon G. *et al*, 2012; Bogaerts P. *et al*, 2016).

6.3. Commercial antibiogram methods

Different commercial antibiogram methods used in routine clinical microbiology laboratory work have been applied directly based on different clinical samples. Commercial strips with an antibiotic gradient have been used to make a direct antibiogram based on respiratory samples. Semi-automated broth micro dilution methods (Micro Scan, VITEK2 and Phoenix) allow the bacteria to be identified and the antibiogram to be obtained directly based on the grown blood culture bottle. The bacteria are identified in 3 h, with poor results in Gram-positive bacteria and

acceptable results in Gram-negative bacteria, and the antibiogram is obtained in 14 h, with good results in both Gram-positive and Gram-negative bacteria (March Rosselló G.A. and Bratos Pérez M.A., 2016).

6.4. Imaging methods

Based on grown blood culture bottles, the ACCELERATE phenol TM SYSTEM apparatus (Accelerate Diagnostics) identifies 10 species and 6 genera of bacteria through the fluorescence in situ hybridisation (FISH) technique in 1 h. To make the direct antibiogram, the bacterial growth of a strain incubated in the presence of different concentrations of antibiotic is monitored through imaging. Thus, in 5 h this piece of equipment reports the MIC and the phenotypes for high-level resistance to gentamicin and streptomycin in enterococci and for induction of clindamycin resistance by erythromycin in staphylococci. Depending on the pathogen studied, the sensitivity obtained has a rate of agreement of 92–100% with that obtained through broth micro dilution (Chantell C., 2015).

6.5. Bacterial lysis methods

Another methodology for determining sensitivity consists of detecting bacterial lysis. To do this, the bacterium is incubated in the presence of the antibiotic at the desired concentration. Then, the bacterium is immobilised in an agarose microgel and exposed to a lysis solution that causes DNA release. Subsequently, the preparation is incubated with the fluorochrome SYBR Gold and the integrity of the DNA may be studied through observation under a fluorescence microscope. This methodology yields an antibiogram in less than 2 h (Santiso R. *et al*, 2011).

7. Antibiotic and Immunity

Infections have been the leading cause of most diseases in the history of mankind (Mondragón *et al.*, 2014). Especially bacterial infections are more prevailing among these. The most common procedure known to fight bacterial infection is through antibiotic therapy applied to individuals. The expression of resistance to antimicrobial agents in this therapy is both the logical and indispensable outcome of using these agents to treat human infections (Ternent *et al.*, 2014). Resistance developed by the bacteria against antibiotics is described as the talent of bacteria to resist the effects of antibiotics generated either to eradicate or control them (Arya, 2007). The release of each new class of antibiotics for treatment, shortly after, has been followed by the emergence of new strains of bacteria which are resistant to this class (Butler and Buss, 2006; Clatworthy *et al*, 2007; Lewis, 2013). In this respect, developing new treatment strategies for bacterial infections is very important (Mondragón *et al*, 2014). According to its properties, antibiotics has the bacteriostatic action to stop the growth of bacteria and bactericidal action to wipe out the bacteria. However, the distinction between these properties is not explicit, as it depends on the drug concentration used and the type and the growth stage of bacteria (Zhang, 2009). Hence, multiple antibiotic therapy is a more appropriate form of treatment. In fact, the bacterial infection is a complicated process for both the infectious bacteria and the host (Carvalho *et al.*, 2012). It is suggested that a significant role in the progress of infections is basically played by the immune system (Linares and Martinez, 2005). The immune system is expressed as a system of biological structures and processes in an organism protecting the body from likely harmful substances via recognizing and responding to antigens (Alberts *et al.*, 2002).

7.1. Bactericidal vs Bacteriostatic the context of Innate Immunity

The few studies on the pharmacodynamics interactions between innate immunity and exogenous antibiotics have revealed differences in the exposure–response relationship. Some antibiotics, including those that have been traditionally labeled "bacteriostatic," such as chloramphenicol and erythromycin, may antagonize the activity of endogenous host defense peptides (Kristian, S. A. *et al*, 2007). In contrast, beta-lactam antibiotics, which are often touted for their overall bactericidal activities, have been shown to further synergize with cationic antimicrobial peptides produced by innate immunity (Sakoulas G. *et al*, 2014). This synergy is so profound that the addition of antistaphylococcal beta-lactams like nafcillin and oxacillin has been successfully deployed as adjunctive salvage therapy to successfully clear refractory MRSA bacteremia (Dhand A. *et al*, 2011; Dhand A. *et al*, 2014). Ampicillin has been used in an analogous fashion to clear persistent bacteremia due to ampicillin-resistant, vancomycinresistant *Enterococcus faecium* (VRE) (Sakoulas G. *et al*, 2012). In both cases, concentrations well below the MIC rendered MRSA and VRE hypersusceptible to cationic antimicrobial peptides, a property completely missed in standard AST testing, which may serve a critical role in the resolution of severe infection. Synergy with antimicrobial peptides has also been shown for ceftaroline against *Streptococcus pneumoniae* (Sakoulas G. *et al*, 2015) and ceftriaxone against *Salmonella enterica* (Sakoulas G. *et al*, 2017). The cationic defense peptide cathelicidin is a key host defense against systemic infection and bacterial meningitis. Antimicrobial synergy with cathelicidin may be an important factor driving better clinical outcomes (Sakoulas G. *et al*, 2017). This enhancement between antibiotics and immune response extends beyond beta-lactams. Azithromycin, a macrolide antibiotic, demonstrates synergy with host antimicrobial peptides against *Pseudomonas* and *Acinetobacter* (Lin L. *et al*, 2015). Even certain

beta-lactamase inhibitors, conceptually deployed to inhibit beta lactam hydrolysis by beta-lactamase enzymes, can themselves act synergistically with endogenous antimicrobial peptides or peptide antibiotics like daptomycin and colistin (Sakoulas G. *et al* ,2017 ; Ulloa E.R. *et al* ,2017).

7.2. Counteracting the negative effect of antibiotics on immunity

Antibiotics can have a negative impact on the microbiota, immunity and health. Avoidance of antibiotics, however, is often not feasible, because many infections can only be survived with antibiotic treatment. For this reason, several strategies have been tested to counteract the negative effect of antibiotics on the microbiota and immune responses. One strategy is to provide probiotics, usually live bacteria, to supplement antibiotic induced deficits in the microbiota. In studies done with streptomycin-treated mice, oral administration of anaerobic microbiota cultures partially prevents Salmonella infection (Ubeda C. & Pamer E.G. , 2012). Several studies in humans have investigated probiotics to prevent and treat infectious disease (Wolvers *et al*, 2010). Administration of a mixture of *Streptococcus thermophilus*, *Lactobacillus casei* and *Lactobacillus bulgaricos* prevents intestinal disease caused by *C. difficile* (Hickson M. *et al*, 2007).

8. Antibiotic Prophylaxis

8.1. What is antibiotic prophylaxis?

Antibiotics usually are used to treat bacterial infections. Sometimes, though, dentists or physicians suggest taking antibiotics before treatment to decrease the chance of infection. This is called **antibiotic prophylaxis**.

During some dental treatments, bacteria from the mouth enter the bloodstream. In most people, the immune system kills these bacteria. There is concern, though, that in some patients, bacteria from the mouth can travel through the bloodstream and cause an infection somewhere else in the body. Antibiotic prophylaxis may offer these people extra protection. (Wilson W. *et al*, 2008).

Preoperative antibiotic prophylaxis is defined as administering antibiotics prior to performing surgery to help decrease the risk of postoperative infections. The evidence supporting routine preoperative use of prophylactic antibiotic administration continues to grow. (AlBuhairan B. *et al*, 2008) The routine administration of prophylactic antibiotics is standard in cases where a patient will have an artificial implant or foreign body implanted as part of the procedure, bone grafting procedures, and other surgeries with extensive dissections or expected high blood loss.

The timing of antibiotic administration may vary, but the goal of administering preoperative systemic prophylactic antibiotics is to have the concentration in the tissues at its highest at the start and during surgery. (W-Dahl A. *et al*, 2011; Tarchini G. *et al*, 2017).

The most common organisms implicated as causes of surgical site infections include (Tan T.L. *et al*, 2017):

- *Staphylococcus aureus*
- *Staphylococcus epidermidis*
- Aerobic streptococci
- Anaerobic cocci

9. Antibiotic and Vaccine

9.1. Vaccine

A vaccine is a biological preparation that improves immunity to a particular disease. A vaccine typically contains an agent that resembles a disease-causing microorganism, and is often made from weakened or killed forms of the microbe, its toxins or one of its surface proteins. The agent stimulates the body's immune system to recognize the agent as foreign, destroy it, and "remember" it, so that the immune system can more easily recognize and destroy any of these microorganisms that it later encounters (Glossary of Immunization and Public Health Terms, 2011).

9.2. What's the Difference between Antibiotics and Vaccines?

The first is timing: antibiotics are generally taken once a bacterial infection has already occurred and the bacterial population is large enough to cause disease. At this stage, the bacteria have already multiplied many times. Each time the bacteria divide their DNA is copied, and mistakes in this process can create variation within the population. This means that by the time a patient takes an antibiotic, the bacterial population is already large and varied enough that a resistant strain is likely to have arisen. It has a greater chance of thriving in this environment since other strains, which it would normally have to compete with, are killed off by the antibiotic. This logic is backed up by studies which show that the larger a microbial population is at the time of treatment, the more likely drug resistance is to evolve. By contrast, vaccines are administered prior to infection. Their role is to prime the immune system to fight any future infections, so that they can be brought under control before the bacteria have had a chance to multiply (Kennedy D.A., 2017).

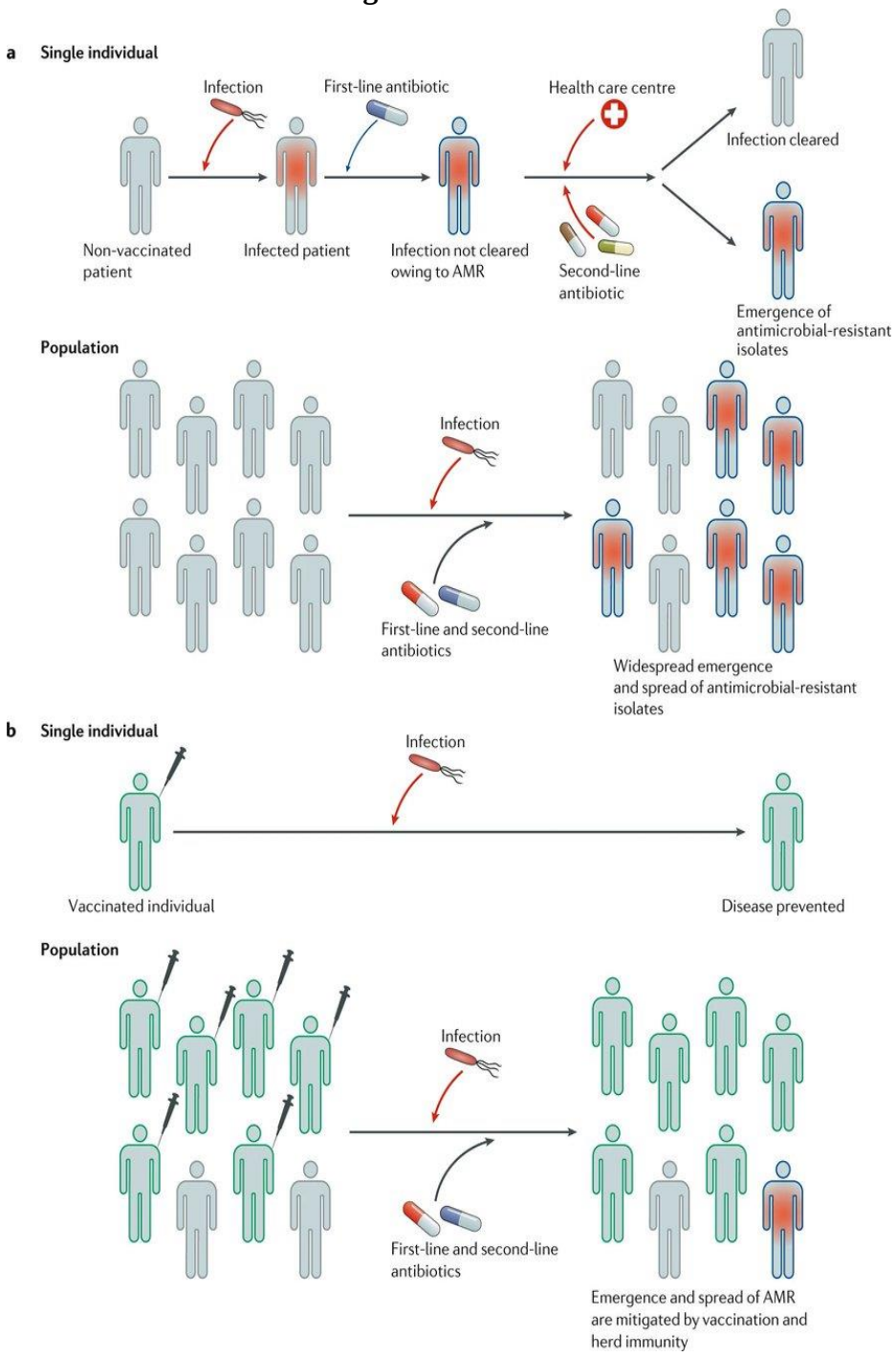
A second difference is the intricacy of how vaccines defend against bacterial infection. Antibiotics usually target one specific bacterial protein or mechanism. In some cases, a single mutation is sufficient to change the target so that the drug does not recognize it anymore, providing resistance to the bacterium. By contrast, some vaccines work by exposing the immune system to a high number of bacterial proteins, leading to the development of a wide range of antibodies. These antibodies will in turn form a complex defense mechanism that prevents bacterial infection and subsequent complications. The chances of the bacteria simultaneously developing resistance to attack from every type of antibody generated following vaccination is rather unlikely (GAVI, The Vaccine Alliance, 2017).

9.3. Direct and indirect effects of vaccines on antimicrobial resistance

Vaccines can reduce the emergence and spread of AMR both directly and indirectly (Klugman K.P. And Black S., 2018; Mishra R.P., *et al*, 2012).**First**, a vaccine against a given bacterial pathogen reduces prevalence of the resistant pathogen as well as antibiotic use. Probably the best documented example of this effect is the pneumococcal vaccine. Several studies suggest that decreased pathogen carriage and infections in vaccines substantially reduced antibiotic prescriptions and diminished the circulation of resistant strains (Lipsitch M. and Siber G.R., 2016). These findings suggest that herd immunity is a key mechanism in reducing the circulation of antimicrobial-resistant pneumococcal strains (Sihvonen R. *et al*, 2017). Also, the introduction of *Haemophilus influenzae* type b conjugate vaccine reduced the need for antibiotics and avoided the continued evolution of resistance, as indicated by data from India, where the introduction of *H. influenzae* type b vaccine was delayed (John T.J., *et al*, 1998).

The veterinary and agricultural settings account for more than 50% of global antibiotic consumption (Oliver S.P., *et al*, 2011), which has been reported to be an important driver of the emergence of resistance (McEwen S.A., 2002). **Recently**, it was demonstrated that use of vaccines in food-producing animals substantially decreased antibiotic use and reduced the risk of the emergence of antibiotic resistance (Hoelzer K. *et al.,* 2018). This might also have implications for human health as resistance determinants might be transferred to bacteria that infect humans or resistant pathogens might infect humans directly. However, more studies are needed to confirm this. Furthermore, vaccinations indirectly affect AMR by preventing viral infections. For example, influenza vaccines can reduce the inappropriate use of antibiotics and prevent secondary bacterial super infections that may occur in a patient who has been infected with the influenza virus (Klugman K.P. & Black S., 2018). In some cases, vaccines have led to the eradication of pathogens, such as the global eradication of smallpox and the animal pathogen rinderpest virus (Hoelzer K. *et al.*, 2018), and the almost complete elimination of poliomyelitis, as well as a decrease of more than 95% in the incidence of diseases such as diphtheria, tetanus, pertussis, measles, mumps and rubella (Mishra R.P., *et al*,2012).Although formal studies to quantify the impact of these vaccines on reducing AMR have not been performed, it is plausible to assume an important contribution through indirect mechanisms by reducing antibiotic use and therefore selection pressure on pathogens. Unfortunately, vaccines against major antimicrobial resistant pathogens are still missing. However, predictions of the impact of vaccines against antimicrobial-resistant pathogens suggest that vaccines could have a substantial impact in controlling resistance (Tekle Y.I. *et al*., 2012).

Figure 9.1. Effects of vaccines on antimicrobial resistance



a | Antimicrobial-resistant bacterial pathogens can cause serious, potentially life-threatening infections in individuals. Treatment with currently available first-line antibiotics is ineffective against resistant infections, and second-line antibiotics may be required to resolve the infection. However, use of the second-line antibiotic may promote the emergence of new antimicrobial-resistant isolates resistant to second-line antibiotics. At the population level, the emergence and spread of antimicrobial resistance (AMR) consequently leads to difficulties in treating patients who are infected. Pathogens resistant to antimicrobials cause substantial morbidity and death. **b** | Vaccines against antimicrobial-resistant pathogens could prevent or reduce life-threatening diseases and thus decrease health care costs, and also reduce the use of antibiotics (both first-line and second line drugs) with the potential of decreasing the emergence of AMR. If sufficient vaccine coverage is achieved in a population, indirect protection (herd immunity) further prevents spread of resistant strains. Decreased disease burden would also negate the need for antibiotics.

9.4. What makes vaccines more effective?

Vaccines contain a variety of features that make them highly successful in the fight against AMR. **First**, aside from the targeted strains, vaccination typically has minimal impact on the development of microbes. This is due to the fact that vaccines function by allowing the immune system to detect antigens that are very specific to the diseases being vaccinated against (Jit, *et al*., 2020). Antimicrobials, on the other hand, can cause both targeted and non-targeted bacteria to acquire resistance. Broad-spectrum antibiotics in particular, interfere with the human microbiome, especially in children, thus affecting the general health (Relman & Lipsitch, 2018). Besides, they have also been shown to enhance selection of resistance in bystander bacterial species of the normal flora (Tedijanto *et al*., 2018). According to Kennedy and Read (2018), vaccine resistance is considerably less likely to emerge than drug resistance. Still, it is more difficult to establish when it occurs, and the molecular basis is less well known. Nonetheless, in the instances studied, the significant health advantages linked with immunization were substantially maintained. Vaccine resistance, it is argued, is less of a worry than medication resistance since it is less likely to emerge and, when it does, is less damaging to human and animal health and well-being. Such cases of vaccine resistance have been reported for *S. pneumoniae, B. pertussis, Yersinia ruckeri*, among others (Kennedy & Read, 2018).

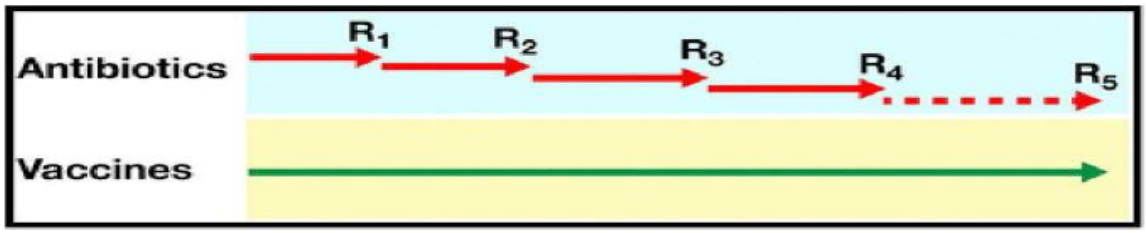


Figure 9.2. The usage of antibiotics selects for resistance (R), rendering the antibiotic useless. As a result, successful therapy necessitates a constant supply of new antibiotics. Vaccines, on the other hand, can be used for a long period without causing considerable resistance

Secondly, due to the particular character of vaccination, vaccines targeting certain strains of a disease that are more harmful or inclined to developing resistance can be created (Jit, *et al.*, 2020). This was the case with *S. pneumoniae* vaccines which were developed using virulence factors that were most likely to trigger aggressive illness (Feldman & Anderson, 2014). **Thirdly**, vaccines and antimicrobial agents can act in tandem, according to (Rynkiewicz, *et al.*, 2016) vaccines can lower the rate at which people become infected, and therefore extending the time it takes for a disease to develop tolerance to a medication. **Finally,** vaccinations can be given merely a few times and have a long-term influence on the entire community by avoiding disease. This is accomplished in part through vaccines' near-lifetime effects (Blok *et al.*, 2015) and in part through the establishment of herd immunity, which prevents the transmission of an infectious agent (Mallory *et al.*, 2018; Rasmussen, 2020). Antimicrobial agents, on the other hand, must be provided on a regular basis in response to each attack. They have limited capacity to halt the forward spread of drug resistant bacteria since there is often a gap between the onset of virulence and receiving treatment (Hobson *et al.*, 2021).

9.5. Pathways by which vaccines can reduce AMR

The potential benefit of vaccines to reduce AMR is frequently underestimated because people only consider a subset of the pathways by which vaccines can affect antimicrobial use and resistance. In total, we consider six pathways by which vaccines can reduce the burden of AMR (Lipsitch M. and Siber G., 2016; Atkins KE. *et al*, 2018).

Pathway 1: Preventing infections by focal pathogens Vaccines may reduce the incidence of infection by a resistant pathogen. This can occur both through direct protection to those vaccinated, and through indirect protection resulting from reduced exposure to the infection in the unvaccinated (herd immunity).

Pathway 2: Bystander effects any vaccines that lead to changes in antibiotic use could potentially have an impact on AMR in organisms not targeted by the vaccine, such as commensal bacterial pathogens, as a result of reduced antibiotic selection pressure. For example, since influenza infections are frequently treated with antibiotics (either inappropriately for the primary viral infection, or for a secondary bacterial infection), an effective and widely used vaccine that reduces the number of influenza infections should result in population-wide reductions in antibiotic use.

Pathway 3: Infection severity effects Vaccines that reduce the risk of symptomatic infection without reducing the risk of carriage/asymptomatic infection can lead to reductions in the proportion of infections which are treated with antimicrobials and therefore a reduction in the selection pressure for resistant phenotypes.

Pathway 4: Subtype selection effects some vaccines may target subtypes of a pathogen population which are more likely to be resistant. As a result, overall resistance may decrease. However, it is also possible that vaccines may target subtypes which are less likely to be resistant. In these circumstances, overall resistance may increase.

Pathway 5: Interspecific effects Bacteria and viruses interact in complex ways. For example, influenza or respiratory syncytial virus (RSV) infections may increase the risk of secondary bacterial infections and patients with certain viral infections may transmit more bacterial pathogens. Vaccination against one organism could therefore reduce transmission of another, leading to declines in both resistant and sensitive phenotypes.

Pathway 6: Selective targeting effects Interventions, such as hygiene improvements or vaccination, could lead to differential effects if targeted to certain population groups. For example, if a resistant strain of a given pathogen transmits preferentially in hospitals (where antibiotic use is high), targeting the hospital population with a vaccine could have a greater overall effect on the resistant strain, leading to declines in resistance in both hospitals and the community.

10. Antibiotic vs Probiotic

Antibiotics, as substances that either prevent the growth of or kill a living organism, are considered miracle drugs. Antibiotics can enhance human lives by treating or preventing diseases. However, a major public-health threat is the resistance to antibiotics or the increased capability of bacteria to stay alive in the presence of antibiotics. Moreover, antibiotics only treat bacterial infections and cannot be effectively used to treat virus-related infections, such as colds (Cars, *et al.*, 2008; Costelloe *et al.*, 2010). Antibiotics have the greatest effect on human health among all medical developments achieved since the beginning of the 20th century. Antibiotics are selective and specific in their target; thus, these drugs can eradicate invading bacteria without inducing toxicity to the infected host (Guarner *et al.*, 2006). Antibiotics are frequently prescribed in most countries (Quigley, 2011).

10.1. Probiotic

Immediately after birth, the human body is colonized by different microorganisms, such as archaea, bacteria, fungi, viruses and microeukaryotes (Aagaard *et al*., 2014). Over time, colonization occurs so intensely that the human microbiome of an adult individual contains more bacterial cells than human cells (Sender Fuchs & Milo, 2016). Different types of microorganisms can cause disease in humans and some of which have a high fatality rate (Peterson, *et al.*, 2009). For many years, scientific research has focused on understanding pathogenic bacteria and finding ways to preventing and treating human diseases. Conversely, some bacterial species may bring benefits to the host through a symbiotic relationship. These microorganisms are generally named probiotics (Fijan, 2014).

Probiotics are living microorganisms (yeast or bacteria) that provide beneficial effects while colonizing the host. Lactic acid bacteria species (*Lactococcus, Lactobacillus, Streptococcus and Enterococcus*) and *Bifidobacterium* (Doron & Snydman, 2015; Prado & de Lindner, 2015; Soccol *et al*, 2015) are among the best-known probiotics. These microorganisms have characteristics that give them the ability to withstand adverse conditions in the host organism, such as enzymatic action and acidity. They can colonize the host and contribute to health by regulating the microbiome and performing biological functions (de Melo Pereira *et al*, 2018).

The probiotics that colonize the human host are most numerous in the intestines. The commensal intestinal microbiome contributes to increased resistance against infections, host immune system differentiation, and synthesis of nutrients (Ubeda & Pamer, 2012). There is evidence that probiotics may act in the treatment and prevention of infectious diseases (Yang *et al.,* 2019). Currently, infectious diseases are commonly managed with the administration of antibiotics. However, an irrational use of antibiotics may cause consequences at the patient level, such as drug-specific adverse effects, and at the public health level, such as selection of multidrug-resistant bacteria (Yang *et al*, 2019). Thus, the search for new alternatives in antimicrobial therapy is much needed, with a special interest in natural product-based therapies (Silva *et al*, 2019).

10.2. Era of Probiotics: The concept of probiotics was first proposed by noble prize winner Elie Metchnikoff in the year 1908. He noticed that the long life of Bulgarian peasants was mainly attributed to their consumption of fermented milk products (Mercenier A. *et al*, 2003; Tannock G. W., 2003). The term probiotics was coined by Lilley and Stillwell in 1965. They referred it as substance secreted by microbes to stimulate the growth of others (Hague R., 2011).

The main biological mechanisms of action of probiotics include increased epithelial barrier, increased adhesion to the intestinal mucosa and inhibition of microbial adhesion and competitive exclusion of pathogenic microorganisms, production of antimicrobial substances and immune system modulation (Bermudez-Brito *et al.*, 2012).

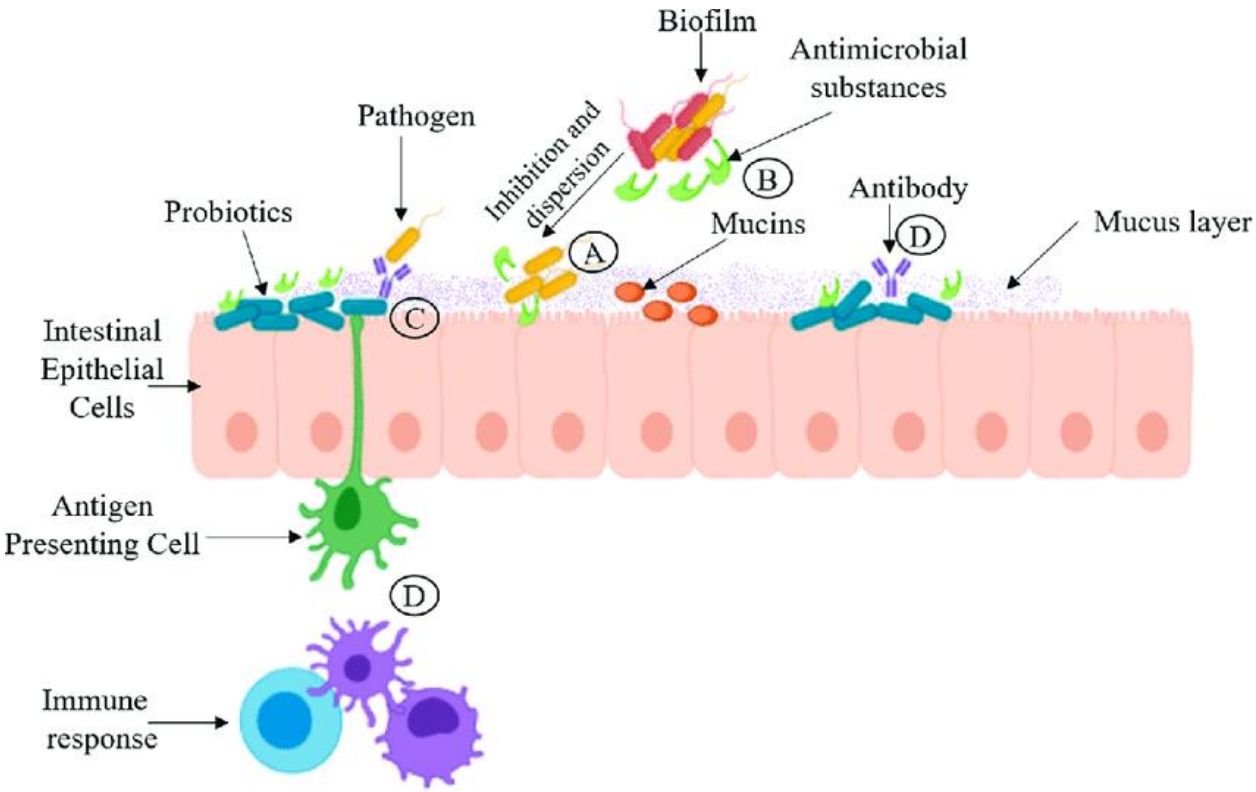


Figure 10. Mechanisms of action of probiotics. (A) Competitive exclusion of pathogenic microorganisms. (B) Production of antimicrobial substances. (C) Increased adhesion to the intestinal mucosa and improvement of the epithelial barrier. (D) Stimulation of the immune system.

10.3. Combination of Antibiotics and Probiotics:

Probiotics enhance the antibiotic therapy as they diminish microbial adhesion and growth by the release of bacteriocins or other inhibitory compounds (Reid G., 2006). In patients on antibiotic regimen probiotics encourage the recovery of commensal microbiota and increase treatment tolerability (Boyanova L. & Mitov I., 2012). Even though probiotics reveals an excellent overall safety profile but should be used cautiously in severely immunocompromised patients due to increased risk of bacteremia (Gupta V. & Garg R., 2009). Antibiotic resistance of the probiotic strains should be kept in mind as it could be transferred to other species, although transfer from lactobacilli has been observed occasionally in in-vivo studies on animal models (Schjorring S. & Krogfelt K.A., 2011). Probiotics reduce the risk of antibiotic-induced superinfections (Boyanova L. & Mitov I., 2012). Adding probiotics to antibiotic regimens of *H.pylori* infection showed a reduction in adverse effects by 11-23% and improved the eradication rate by approximately 5-15% (Lesbros-Pantoflickova D. *et al*, 2007; Boyanova L., 2011). Even though few combinations of antibiotics and probiotics are available in the market, but their effectiveness is questionable. There are controversies regarding incorporation of antibiotics and probiotics in the same drug. It is advisable to stagger the administration of the antibiotic and probiotic such that the probiotic is administered at least three hours following the antibiotic dose, where possible, or else the antibiotic may lessen the efficacy of the probiotic microorganisms. But the reverse of this is not true, i.e., probiotics do not reduce the potency or effectiveness of antibiotics. Selection of probiotic strains, their appropriate dosage and prolong consumption are important for long-term benefits. Usually, the administration of probiotics on patients undergoing antibiotic therapy is 1-3 weeks longer than the time period of antibiotic treatment (Biradar S. S. *et al*, 2005; Szajewska H. *et al*, 2010).

10.4. Use of Probiotics and Their Impact on Microbiota in Infection Diseases

Probiotics, frequently described as good bacteria, are commonly found in foods or consumed as dietary supplements or as a replacement for native gut bacteria (Kaur H. & Ali S.A., 2022). They work in competition with other species of pathogenic or non-pathogenic bacteria (Knipe H. *et al*, 2021) Most of their metabolites negatively impact the growth of other bacterial species or strains (Hibbing M.E. *et al*, 2010).Probiotics are hypothesized to restore the altered intestinal microbiome and may provide health benefits through three main mechanisms:

(1) By the inhibition of pathogen growth.

(2) By the replacement of pathogenic bacteria.

(3) By the creation of a more favorable microbial environment in the stomach and gut. It is well established that probiotics can reduce the frequency of certain infections and attenuate the symptoms of such infections (Tung J. M. *et al*, 2009). For instance, using probiotics in intubated critically ill patients is as efficient as using selective digestive decontamination with antibiotics in reducing secondary infections (Batra P., *et al*, 2020). Further, the use of probiotics for infection control and prophylaxis is currently a very important complement to the standard treatment of infection (Jeppsson B. *et al*, 2011).

References

- Aagaard, K., Ma, J., Antony, K. M., Ganu, R., Petrosino, J., & Versalovic, J. (2014). The placenta harbors a unique microbiome. Science Translational Medicine, 6, 237ra65.
- Adzitey F. (2015). Antibiotic classes and antibiotic susceptibility of bacterial isolates from selected poultry; a mini review. World Vet. J. 5 (3):36-41.
- Alberts B., Johnson A. and Lewis J. *et al* (2002) Molecular biology of the cell. In: The adaptive immune system, 4th edn. Garland Science, New York. ISBN 10: 0-8153-3218-1.
- AlBuhairan B., Hind D., Hutchinson A. (2008). Antibiotic prophylaxis for wound infections in total joint arthroplasty: a systematic review. J Bone Joint Surg Br. 90(7):915-9.
- Althani, A. A., Marei, H. E., Hamdi, W. S., Nasrallah, G. K., El Zowalaty, M. E., Al Khodor, S., ... & Cenciarelli, C. (2016). Human microbiome and its association with health and diseases. *Journal of cellular physiology*, *231*(8), 1688-1694.
- Aminov R. I. (2010). A brief history of the antibiotic era: Lessons learned and challenges for the future. Front Microbiol. 1(134):1-7.
- Anderson D.J., Engemann J.J., Harrell L.J., Carmeli Y., Reller L.B., Kaye K.S. (2006).Predictors of mortality in patients with bloodstream infection due to ceftazidime-resistant Klebsiella pneumoniae. Antimicrob Agents Chemother.50 (5):1715-20.
- Arya D.P. (2007) Aminoglycoside antibiotics: from chemical biology to drug discovery. Wiley, New Jersey.
- Atkins K.E., Lafferty E.I., Deeny S.R., *et al.* (2018).Use of mathematical modelling to assess the impact of vaccines on antibiotic resistance. Lancet Infect; 18(6):e204–13.
- Avent M.L., Rogers B.A., Cheng A.C., Paterson D.L. (2011).Current use of aminoglycosides: indications, pharmacokinetics and monitoring for toxicity. Intern Med J.41 (6):441-9.
- Batra, P., Soni, K. D., & Mathur, P. (2020). Efficacy of probiotics in the prevention of VAP in critically ill ICU patients: an updated systematic review and meta-analysis of randomized control trials. *Journal of intensive care*, *8*(1), 1-14.
- Bébéar C.M., Pereyre S. (2005).Mechanisms of drug resistance in *Mycoplasma pneumoniae*. *Curr Drug Targets.*5:263–271.
- Beceiro A., Tomás M., Bou G. (2013) Antimicrobial resistance and virulence: a successful or deleterious association in the bacterial world? Clin Microbiol Rev 26: 185–230.
- Belizário, J. E., & Napolitano, M. (2015). Human microbiomes and their roles in dysbiosis, common diseases, and novel therapeutic approaches. *Frontiers in microbiology*, *6*, 1050.
- Bell, G., & MacLean, C. (2018). The search for 'evolution-proof'antibiotics. *Trends in Microbiology*, *26*(6), 471-483.
- Bermudez-Brito, M., Plaza-Díaz, J., Muñoz-Quezada, S., Gómez-Llorente, C., & Gil, A. (2012). Probiotic mechanisms of action. Annals of Nutrition & Metabolism, 61, 160–174.
- Biradar, S. S., Bahagvati, S. T., & Shegunshi, B. (2005). Probiotics and antibiotics: a brief overview. *Internet J Nutr Wellness*, *2*(1), 1-6.
- Blair J.M., Richmond G.E., Piddock L.J. (2014).Multidrug efflux pumps in Gram-negative bacteria and their role in antibiotic resistance. *Future Microbiol.* 9:1165–1177.

- Blair J.M., Webber M.A., Baylay A.J., *et al*. (2015) Molecular mechanisms of antibiotic resistance. Nat Rev Microbiol 13: 42–51.
- Blok B.A., Arts R.J.W., van Crevel R., Benn C.S., Netea M.G. (2015).Trained innate immunity as underlying mechanism for the long-term, nonspecific effects of vaccines. *J. Leukoc. Biol.;* 98(3):347–356.
- Bogaerts P., Cuzon G., Evrard S., Hoebeke M., Naas T., Glupczynski Y. (2016). Evaluation of a DNA microarray for rapid detection of the most prevalent extended-spectrum beta-lactamases, plasmid-mediated cephalosporinases and carbapenemases in Enterobacteriaceae, Pseudomonas and Acinetobacter. Int J Antimicrob Agents.; 48:189–93.
- Boyanova, L. (2011). Non-antibiotic agents in the treatment of H. pylori infection. *Helicobacter Pylori, L. Boyanova, Ed*, 253-275.
- Boyanova, L., & Mitov, I. (2012). Coadministration of probiotics with antibiotics: why, when and for how long?. *Expert review of anti-infective therapy*, *10*(4), 407-409.
- Brooks G. F., Butel J. S. & Morse S. A. (2004). Jawetz, Melnick and Adelberg‟s Medical Microbiology, 23rd Edition. McGraw Hill Companies, Singapore.
- Brooks G.F., Carroll K.C., Butel J.S., Morse S.A., Mietzner T.A. (2013). Antimicrobial chemotherapy. In: Jawtz, Melnick & Adelberg's Medical Microbiology. USA, McGraw-Hill companies Inc. 26th edn. p 371-405.
- Butler M.S. , Buss A.D. (2006) Natural products the future scaffolds for novel antibiotics? Biochem Pharmacol 71(7):919–929.
- C. C. Brackett, H. Singh, J. H. Block, (2004). Likelihood and mechanisms of cross-allergenicity between sulfonamide antibiotics and other drugs containing a sulfonamide functional group, Pharmacotherapy, 24(7) 856-870.
- Calderon C. B. & Sabundayo B. P. (2007). Antimicrobial classifications: Drugs for bugs. In: Schwalbe R, Steele-Moore L & Goodwin AC (eds). Antimicrobial susceptibility testing protocols. CRC Press, Taylor and Frances group. ISBN 978-0-8247-4100-6.
- Carter B.L., Woodhead J.C., Cole K.J., Milavetz G. (1987).Gastrointestinal side effects with erythromycin preparations. Drug Intell Clin Pharm. 21(9):734-8.
- Carvalho R.V., Kleijn J. and Meijer A. *et al* (2012) Modeling innate immuneresponse to early mycobacterium infection. Compute Math Methods Med 790482:1–12.
- Chancey S.T., Zähner D., Stephens D.S. (2012).Acquired inducible antimicrobial resistance in Gram-positive bacteria. *Future Microbiol.* ; 7:959–978.
- Chantell C. (2015) Multiplexed automated digital microscopy for rapid identification and antimicrobial susceptibility testing of bacteria and yeast directly from clinical samples. Clin Microbiol Newsl. 37:161–7.
- Chopra I. & Roberts M. (2001). Tetracycline antibiotics: Mode of action, applications, molecular biology, and epidemiology of bacterial resistance. Microbiol. Mol. Biol. Rev. 65(2):232-260.
- Choquet-Kastylevsky G., Vial T. & Descotes J. (2002). Allergic adverse reactions to sulfonamides. Curr. Allergy Asthma Rep. 2(1):16-25.
- Clatworthy A.E., Pierson E.P. and Hung D.T. (2007) Targeting virulence: a new paradigm for antimicrobial therapy. Nat Chem Biol 3:541–548.

- Cohen A.L., Calfee D., Fridkin S.K. *et al*. (2008). Recommendations for metrics for multidrug-resistant organisms in healthcare settings: SHEA/ HICPAC Position paper. Infect Control Hosp Epidemiol 29: 901- 913.
- Collignon, P. C., Conly, J. M., Andremont, A., McEwen, S. A., Aidara-Kane, A., World Health Organization Advisory Group, Bogotá Meeting on Integrated Surveillance of Antimicrobial Resistance (WHO-AGISAR), ... & Woo, G. J. (2016). World Health Organization ranking of antimicrobials according to their importance in human medicine: a critical step for developing risk management strategies to control antimicrobial resistance from food animal production. *Clinical Infectious Diseases*, *63*(8), 1087-1093.
- Cornaglia G., Mazzariol A., Fontana R., *et al.* (1996).Diffusion of carbapenems through the outer membrane of enterobacteriaceae and correlation of their activities with their periplasmic concentrations. *Microb Drug Resist.* ; 2:273–276.
- Cosgrove S.E., Sakoulas G., Perencevich E.N., Schwaber M.J., Karchmer A.W., Carmeli Y. (2003).Comparison of mortality associated with methicillin-resistant and methicillinsusceptible Staphylococcus aureus bacteremia: a meta-analysis. Clin Infect Dis.36 (1):53-9.
- Costa S.S., Viveiros M., Amaral L., *et al.* (2013) Multidrug efflux pumps in Staphylococcus aureus: an update. Open Microbiol J 7: 59–71.
- Cox G., Wright G.D. (2013). Intrinsic antibiotic resistance: mechanisms, origins, challenges and solutions. Int J Med Microbiol 303: 287–292.
- Cuzon G., Naas T., Bogaerts P., Glupczynski Y., Nordmann P. (2012). Evaluation of a DNA microarray for the rapid detection of extended-spectrum beta-lactamases (TEM, SHV and CTX-M), plasmid-mediated cephalosporinases (CMY-2-like, DHA, FOX, ACC-1, ACT/MIR and CMY-1-like/MOX) and carbapenemases (KPC, OXA-48, VIM, IMP and NDM). J Antimicrob Chemother.67:1865–9.
- De Melo Pereira, G. V., de Oliveira Coelho, B., Magalhães Júnior, A. I., Thomaz-Soccol, V., & Soccol, C. R. (2018). How to select a probiotic? A review and update of methods and criteria. Biotechnology Advances, 36, 2060–2076.
- Denyer S. P., Hodges N. A. & German S. P. (2004). Introduction to pharmaceutical microbiology. In: Denyer SP, Hodges NA & German SP (eds.) Hugo and Russell's Pharmaceutical Microbiology. 7th Ed. Blackwell Science, UK. Pp. 3-8.
- Dhand, A., & Sakoulas, G. (2014). Daptomycin in combination with other antibiotics for the treatment of complicated methicillin-resistant Staphylococcus aureus bacteremia. *Clinical Therapeutics*, *36*(10), 1303-1316.
- Dhand, A., Bayer, A. S., Pogliano, J., Yang, S. J., Bolaris, M., Nizet, V., ... & Sakoulas, G. (2011). Use of antistaphylococcal β-lactams to increase daptomycin activity in eradicating persistent bacteremia due to methicillin-resistant Staphylococcus aureus: role of enhanced daptomycin binding. *Clinical infectious diseases*, *53*(2), 158-163.
- Domagala J. M. (1994). Structure-activity and structure-side-effect relationships for the quinolone antibacterials. J. Antimicrob. Chemother. 33:685-706.
- Doron, S., & Snydman, D. R. (2015). Risk and safety of probiotics. Clinical Infectious Diseases, 60, S129–S134.
- Džidic S., Šuškovic J., Kos B. (2008).Antibiotic resistance mechanisms in bacteria: Biochemical and genetic aspects. Food Technol Biotechnol; 46:11-21.

- Eckburg, P. B., Lister, T., Walpole, S., Keutzer, T., Utley, L., Tomayko, J., & Coleman, S. (2019). Safety, tolerability, pharmacokinetics, and drug interaction potential of SPR741, an intravenous potentiator, after single and multiple ascending doses and when combined with β-lactam antibiotics in healthy subjects. *Antimicrobial agents and chemotherapy*, *63*(9), e00892-19.
- Eliopoulos G.M., Maragakis L.L., Perl T.M. (2008).Acinetobacter baumannii: Epidemiology, Antimicrobial Resistance, and Treatment Options. Clin Infect Dis; 46:1254-1263.
- Exner M., Bhattacharya S., and Christiansen B., *et al.* (2017).Antibiotic resistance: what is so special about multidrug-resistant gram-negative bacteria? GMS Hyg Infect Control.12:Doc05.
- Eyssen H. J., Van den Bosch J. F., Janssen G. A. & Vanderhaeghe H. (1971). Specific inhibition of cholesterol absorption by sulfaguanidine. Atherosclerosis. 14 (2):181-192.
- Falagas M.E., Koletsi P.K., Bliziotis I.A. (2006). The diversity of definitions of multidrug-resistant (MDR) and pandrugresistant (PDR) Acinetobacter baumannii and Pseudomonas aeruginosa. J Med Microbiol. 55: 1619-1629.
- Farzana A. (2013).Detection of bla-NDM1 from imipenem resistant Pseudomonas aeruginosa isolated from burn unit of DMCH & invitro evaluation of different antibiotic combination. Department of Microbiology, Dhaka Medical College, January, [M.Phil thesis].
- Feldman C., Anderson R. (2014).Current and new generation pneumococcal vaccines. *J. Infect.* ; 69(4):309–325.
- Ferri, M., Ranucci, E., Romagnoli, P., & Giaccone, V. (2017). Antimicrobial resistance: A global emerging threat to public health systems. *Critical reviews in food science and nutrition*, *57*(13), 2857-2876.
- Fijan, S. (2014). Microorganisms with claimed probiotic properties: An overview of recent literature. International Journal of Environmental Research and Public Health, 11, 4745–4767.
- Frank U. & Tacconelli E. (2012). The Daschner Guide to In-Hopsital Antibiotic Therapy. European standards. Available online at: http://www.springer.com/978-3-642-18401-7. 300p.
- Friedman, N. D., Temkin, E., & Carmeli, Y. (2016). The negative impact of antibiotic resistance. *Clinical Microbiology and Infection*, *22*(5), 416-422.
- Fuoco D. (2012). Classification framework and chemical biology of tetracycline-structure-based drugs. Antibiotics. 1:1-13.
- GAVI, (2017). The Vaccine Alliance, https://www.gavi.org/vaccineswork/why-do-vaccines-work-against-antibiotic-resistance .
- Gilbert D. (2000). Aminoglycosides. In: Mandell G. L., Bennett J. E. & Dolin R, (eds.) Mandell, Douglas, and Bennett's principles and practice of infectious diseases. 5th ed. Philadelphia: Churchill Livingstone. Pp. 307-336.
- Gill M.J., Simjee S., Al-Hattawi K., *et al*. (1998).Gonococcal resistance to β-lactams and tetracycline involves mutation in loop 3 of the porin encoded at the *penB* locus. *Antimicrob Agents Ch.* 42:2799–2803.
- Glossary of Immunization and Public Health Terms, DOH 348-269 November 2011.
- Gold HS, Moellering R.C. J.r. (1996).Antimicrobial drug resistance. N. Engl J. Med.
- Grundmann H., Aires-de-Sousa M., Boyce J., Tiemersma E. (2006). Emergence and resurgence of meticillin-resistant Staphylococcus aureus as a public-health threat. Lancet; 368:874-85.

- Gupta, V., & Garg, R. (2009). Probiotics. *Indian journal of medical microbiology*, *27*(3), 202-209.
- Hague, R. (2011). What is the threat from extended spectrum β-lactamase-producing organisms in children? *Archives of disease in childhood*, *96*(4), 325-327.
- Hamilton-Miller J. M. (1973). Chemistry and biology of the polyene macrolide antibiotics. Am. Soc. Microbiol. 37(2):166-196.
- Hashempour-Baltork, F., Hosseini, H., Shojaee-Aliabadi, S., Torbati, M., Alizadeh, A. M., & Alizadeh, M. (2019). Drug resistance and the prevention strategies in food borne bacteria: An update review. *Advanced pharmaceutical bulletin*, *9*(3), 335.
- Hauser A.R., (2015).editor. Cell envelope. In: Antibiotic Basic for Clinicians. 2nd Ed. New Delhi: Wolters Kluwer (India) Pvt. Ltd. p. 3-5.
- Hawkey P.M. (2003) Mechanisms of quinolone action and microbial response. J Antimicrob Chemoth 1: 28–35.
- Heesemann J. (1993). Mechanisms of resistance to beta-lactam antibiotics. Infection. 21(1):S4-9.
- Henry R. J. (1943). The mode of action of sulfonamides. Bacteriol. Rev. 7(4):175-262.
- Hibbing, M. E., Fuqua, C., Parsek, M. R., & Peterson, S. B. (2010). Bacterial competition: surviving and thriving in the microbial jungle. *Nature reviews microbiology*, *8*(1), 15-25.
- Hickson, M., D'Souza, A. L., Muthu, N., Rogers, T. R., Want, S., Rajkumar, C., & Bulpitt, C. J. (2007). Use of probiotic Lactobacillus preparation to prevent diarrhoea associated with antibiotics: randomised double blind placebo controlled trial. *Bmj*, *335*(7610), 80.
- Hidron A.I., Edwards J.R., and Patel J. *et al.* (2008).NHSN annual update: antimicrobial-resistant pathogens associated with healthcare-associated infections: annual summary of data reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention, 2006-2007. Infect Control Hosp Epidemiol 29: 996- 1011.
- Higgins P.G., Fluit A.C., Schmitz F.J. (2003).Fluoroquinolones: Structure and target sites. Curr Drug Targets; 4:181-90.
- Hobson C., Chan A.N., Wright G.D. (2021).The antibiotic resistome: A guide for the discovery of natural products as antimicrobial agents. *Chem. Rev.;* 121(6):3464–3494.
- Hoelzer, K. *et al.* (2018). Vaccines as alternatives to antibiotics for food producing animals. Part 1: challenges and needs. Vet. Res. 49, 64.
- Hoelzer, K. *et al.* (2018).Vaccines as alternatives to antibiotics for food producing animals. Part 1: challenges and needs. Vet. Res. 49, 64 .
- Holten K.B., Onusko E.M. (2000) Appropriate prescribing of oral beta-lactam antibiotics. Am Fam Physician.01; 62 (3):611-20.
- Ibrahim E.H., Sherman G., Ward S., Fraser V.J., Kollef M.H. (2000).The influence of inadequate antimicrobial treatment of bloodstream infections on patient outcomes in the ICU setting. Chest.118 (1):146-55.
- Jawetz E., Melnick J.L., Adelberg E.A. (1995).Medical Microbiology. East Norwalk, CT: Appleton & Lange, pp 137-167.
- Jeppsson, B., Mangell, P., & Thorlacius, H. (2011). Use of probiotics as prophylaxis for postoperative infections. *Nutrients*, *3*(5), 604-612.

- Jit, M., Anderson, M., Cooper, B., (2020). Quantifying the benefits of vaccines in combating antimicrobial resistance. Eurohealth 26 (1), 16–19.
- Jit, M., Cooper, B., (2020). The role of vaccines in combating antimicrobial resistance. Eur. J. Public Health, 30(Supplement_5), ckaa165-1204.
- Jo I., Hong S., Lee M., *et al*. (2017) Stoichiometry and mechanistic implications of the MacAB TolC tripartite efflux pump. Biochem Bioph Res Co 494: 668–673.
- John, T.J., Cherian, T., & Raghupathy, P. (1998). Haemophilus influenzae disease in children in India: a hospital perspective. *The Pediatric infectious disease journal*, *17*(9), S169-S171.
- Johnston N.J., Mukhtar T.A., Wright G.D. (2002).Streptogramin antibiotics: Mode of action and resistance. Curr Drug Targets; 3:335-44.
- Kahne D., Leimkuhler C., Lu W., Walsh C. (2005).Glycopeptide and lipoglycopeptide antibiotics. Chem Rev; 105:425-48.
- Kang C.I., Kim S.H., Park W.B., *et al.* (2005). Bloodstream infections caused by antibiotic-resistant gram-negative bacilli: risk factors for mortality and impact of inappropriate initial antimicrobial therapy on outcome. Antimicrob Agents Chemother.49 (2):760–766. Doi: 10.1128/ AAC.49.2.760-766.2005.
- Kaur, H., & Ali, S. A. (2022). Probiotics and gut microbiota: Mechanistic insights into gut immune homeostasis through TLR pathway regulation. *Food & Function*.
- Kennedy D.A., Read A.F. (2018).Why the evolution of vaccine resistance is less of a concern than the evolution of drug resistance. *Proc. Natl. Acad. Sci.; 115*(51):12878–12886.
- Kennedy, D. A. & Read, A. F. (2017).Why does drug resistance readily evolve but vaccine resistance does not? *Proc. R. Soc. B Biol. Sci.* 284.
- Klugman, K. P. & Black, S. (2018). Impact of existing vaccines in reducing antibiotic resistance: Primary and secondary effects. Proc. Natl Acad. Sci. USA 115, 12896–12901.
- Knipe, H., Temperton, B., Lange, A., Bass, D., & Tyler, C. R. (2021). Probiotics and competitive exclusion of pathogens in shrimp aquaculture. *Reviews in Aquaculture*, *13*(1), 324-352.
- Konstantopoulou, A., (2016). "Systematic study and investigation of use and misuse of antibiotics in public health. Interdepartmental postgraduate training." Program of Medicinal Chemistry,
- Kourtesi C., Ball A.R., Huang Y.Y., *et al.* (2013) Microbial efflux systems and inhibitors: approaches to drug discovery and the challenge of clinical implementation. Open Microbiol J 7: 34–52.
- Kristian, S. A., Timmer, A. M., Liu, G. Y., Lauth, X., Sal-Man, N., Rosenfeld, Y., ... & Nizet, V. (2007). Impairment of innate immune killing mechanisms by bacteriostatic antibiotics. *The FASEB Journal*, *21*(4), 1107-1116.
- Kumar A., Schweizer H.P. (2005).Bacterial resistance to antibiotics: active efflux and reduced uptake. *Adv Drug Deliver Rev.* 57:1486–1513.
- Kumar S., Mukherjee M.M., Varela M.F. (2013) Modulation of bacterial multidrug resistance efflux pumps of the major facilitator superfamily. Int J Bacteriol.
- Kuo L.C., Teng L.J., Yu C.J., Ho S.W., Hsueh P.R. (2004).Dissemination of a clone of unusual phenotype of pandrug-resistant Acinetobacter baumannii at a university hospital in Taiwan. J Clin Microbiol 42: 1759-1763.

- Kuo L.C., Yu C.J., Lee L.N. *et al*. (2003). Clinical features of pandrug-resistant Acinetobacter baumannii bacteremia at a university hospital in Taiwan. J Formos Med Assoc 102: 601-606.
- Lambert P.A. (2002).Cellular impermeability and uptake of biocides and antibiotics in gram-positive bacteria and mycobacteria. *J Appl Microbiol.* 92:46S–54S.
- Lambert P.A. (2005).Bacterial resistance to antibiotics: Modified target sites. Adv Drug Deliv Rev; 57:1471-85.
- Leonard, H., Colodner, R., Halachmi, S., & Segal, E. (2018). Recent advances in the race to design a rapid diagnostic test for antimicrobial resistance. *ACS sensors*, *3*(11), 2202-2217.
- Lesbros-Pantoflickova, D., Corthesy-Theulaz, I., & Blum, A. L. (2007). Helicobacter pylori and probiotics. *The Journal of nutrition*, *137*(3), 812S-818S.
- Lewis K. (2013) Platforms for antibiotic discovery. Nat Rev Drug Discov 12:371–387.
- Lin, L., Nonejuie, P., Munguia, J., Hollands, A., Olson, J., Dam, Q., & Nizet, V. (2015). Azithromycin synergizes with cationic antimicrobial peptides to exert bactericidal and therapeutic activity against highly multidrug-resistant gram-negative bacterial pathogens. *EBioMedicine*, *2*(7), 690-698.
- Linares J., Martinez J. (2005) Resistencia a los antimicrobianos y virulencia bacteriana. Enferm Infecc Microbiol Clin 23(2):86–93.
- Lipsitch M., Siber G. (2016).How can vaccines contribute to solving the antimicrobial resistance problem? MBio; 7(3):e00428.
- Lockhart S.R., Abramson M.A., Beekmann S.E., *et al.* (2007).Antimicrobial resistance among gram-negative bacilli causing infections in intensive care unit patients in the United States between 1993 and 2004. J. Clin Microbiol. 45(10):3352–3359. doi:10.1128/JCM.01284- 07
- Ludovici L.J. (1952) *Fleming: Discoverer of Penicillin.* London: The Scientific Book Club.
- Magiorakos A.P., Srinivasan A., and Carey R.B., *et al.* (2012) .Multidrug-resistant, extensively drug resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect.18:268–281. Doi: 10.1111/ j.1469-0691.2011.03570.x.
- Mah T.F. (2012).Biofilm-specific antibiotic resistance. *Future Microbial.* 7:1061–1072.
- Mahajan G.B. & Balachandran L. (2012). Antibacterial agents from actinomycetes - a review. Front Biosci. (Elite Ed). 4:240-253.
- Mahmoud A.G., Rice L.B. (1999) Antifungal agents: mode of action, mechanisms of resistance, and correlation of these mechanisms with bacterial resistance, and correlation. Clin Microbiol Rev 12(4):501–517.
- Mahon C.R., Lehman D.C., Manuselis G. (2014). *Textbook of Diagnostic Microbiology.* St. Louis: Saunders. Antimicrobial agent mechanisms of action and resistance; pp. 254–273.
- Mallory M.L., Lindesmith L.C., Baric R.S. (2018).Vaccination-induced herd immunity: successes and challenges. *J. Allergy Clin. Immunol.* ; 142(1):64–66.
- Mandell G.L., Bennett J.E., Dolin R. Mandell, Douglas, and Bennett's (2000).Principles and practice of infectious diseases. 5thed. Philadelphia: Churchill Livingstone, 404–23.
- March Rosselló G.A., Bratos Pérez M.A. (2016). Antibiograma rápido en Microbiología Clínica. Enferm Infecc Microbiol Clin. 34:61–8.

- Mayer K.H., Opal S.M., Medeiros A.A., (1995).Mechanisms of antibiotic resistance. In Principles and Practice of Infectious Diseases. Ed by GL Mandell, JE Bennett, R Dolin. Mandell, Douglas, and Bennett Õs Fourth Ed. New York: Churchill Livingstone, pp 212-225.
- McAdams, D., Wollein Waldetoft, K., Tedijanto, C., Lipsitch, M., & Brown, S. P. (2019). Resistance diagnostics as a public health tool to combat antibiotic resistance: A model-based evaluation. *PLoS biology*, *17*(5), e3000250.
- McEwen, S. A. & Fedorka-Cray, P. J. (2002). Antimicrobial use and resistance in animals. Clin. Infect. Dis. 34 (Suppl. 3), S93–S106.
- McGeer A., Fleming C. A., Gree K. & Low D. E. (2001). Antimicrobial resistance in Ontario: Are we making progress? Laboratory Proficiency Testing Program Newsletter. 293:1-2.
- Medical News Today (2015). Antibiotics: How do antibiotics work? MediLexicon International Ltd. Bexhill-on-sea UK.
- Mercenier, A., Pavan, S., & Pot, B. (2003). Probiotics as biotherapeutic agents: present knowledge and future prospects. *Current pharmaceutical design*, *9*(2), 175-191.
- Miller W.R., Munita J.M., Arias C.A. (2014).Mechanisms of antibiotic resistance in enterococci. *Expert Rev Anti-Infe.* 12:1221–1236.
- Miller, C. P., & Bohnhoff, M. (1963). Changes in the mouse's enteric microflora associated with enhanced susceptibility to Salmonella infection following streptomycin treatment. *The Journal of infectious diseases*, 59-66.
- Mishra N.N., Bayer A.S., Weidenmaier C., *et al.* (2014) Phenotypic and genotypic characterization of daptomycin-resistant methicillin-resistant Staphylococcus aureus strains: relative roles of mprF and dlt operons. PLoS One 9: e107426.
- Mishra, R.P., Oviedo-Orta, E., Prachi, P., Rappuoli, R. & Bagnoli, F. (2012). Vaccines and antibiotic resistance. Curr. Opin. Microbiol. 15, 596–602.
- Mondragón E.I. , Mosquera S. and Cerón M. *et al* (2014) Mathematical modeling on bacterial resistance to multiple antibiotics caused by spontaneous mutations. BioSystems 117:60–67.
- Moore D. (2015). Antibiotic Classification and Mechanism.
- Morgan, X. C., Tickle, T. L., Sokol, H., Gevers, D., Devaney, K. L., Ward, D. V., & Huttenhower, C. (2012). Dysfunction of the intestinal microbiome in inflammatory bowel disease and treatment. *Genome biology*, *13*(9), 1-18.
- Nikaido H. (2009).Multidrug resistance in bacteria. Annu Rev Biochem; 78:119-146.
- Oliver, S. P., Murinda, S. E. & Jayarao, B. M. (2011).Impact of antibiotic use in adult dairy cows on antimicrobial resistance of veterinary and human pathogens: a comprehensive review. Foodborne Pathog. Dis. 8, 337–355.
- O'Neill, J. (2016). Tackling drug-resistant infections globally: final report and recommendations.
- Pegler S. & Healy B. (2007). In patients allergic to penicillin, consider second and third generation cephalosporins for life threatening infections. BMJ. 335(7627): 991.
- Peterson L. R. (2008). Currently available antimicrobial agents and their potential for use as monotherapy. Clin Microbial. Infect. 14(6):30-45.
- Peterson, J.., et al. (2009). NIH HMP Working Group the NIH Human Microbiome Project. Genome Research, 19, 2317–2323. https://doi.org/10.1101/gr.096651.109.

- Piddock L.J. (2006) clinically relevant chromosomally encoded multidrug resistance efflux pumps in bacteria. Clin Microbiol Rev 19: 382–402.
- Poirel L., Brinas L., Verlinde A., Ide L. & Nordmann P. (2005). BEL-1, a novel clavulanic acid-inhibited extended-spectrum beta-lactamase, and the class 1 integron In120 in Pseudomonas aeruginosa. Antimicrob Agents Chemother. 49(9):3743-3748.
- Poole K. (2007) Efflux pumps as antimicrobial resistance mechanisms. Ann Med 39: 162–176.
- Prado, F. C., Lindner, J. de D., Inaba, J., Thomaz-Soccol, V., Brar, S. K., & Soccol, C. R. (2015). Development and evaluation of a fermented coconut water beverage with potential health benefits. Journal of Functional Foods, 12, 489–497.
- R. S. Gruchalla, (1999). Diagnosis of allergic reactions to sulfonamides, Allergy, 54 28-32.
- Rahal J.J. (2016).Novel Antibiotic Combinations against Infections with Almost Completely Resistant *Pseudomonas aeruginosa* and *Acinetobacter* Species. Clin Infect Dis 63:S95-S98.
- Ramirez M.S., Tolmasky M.E. (2010) Aminoglycoside modifying enzymes. Drug Resist Update 13: 151–171.
- Randall C.P., Mariner K.R., Chopra I., *et al*. (2013) The target of daptomycin is absent form Escherichia coli and other gram-negative pathogens. Antimicrob Agents Ch 57: 637–639.
- Rasmussen A.L. (2020).Vaccination is the only acceptable path to herd immunity. *Med.;* 1(1):21–23.
- Redgrave L.S., Sutton S.B., Webber M.A., *et al.* (2014). Fluoroquinolone resistance: mechanisms, impact on bacteria, and role in evolutionary success. Trends Microbiol 22: 438–445.
- Reid, G. (2006). Probiotics to prevent the need for, and augment the use of, antibiotics. *Canadian Journal of Infectious Diseases and Medical Microbiology*, *17*(5), 291-295.
- Relman, D.A., Lipsitch, M., (2018). Microbiome as a tool and a target in the effort to address antimicrobial resistance. Proc. Natl. Acad. Sci. 115 (51), 12902–12910.
- Reygaert W.C. (2009) Methicillin-resistant Staphylococcus aureus (MRSA): molecular aspects of antimicrobial resistance and virulence. Clin Lab Sci 22: 115–119.
- Roberts M.C. (2003) Tetracycline therapy: update. Clin Infect Dis 36: 462–467.
- Roberts M.C. (2004) Resistance to macrolide, lincosamide, streptogramin, ketolide, and oxazolidinone antibiotics. Mol Biotechnol 28: 47–62.
- Roberts R.R., Hota B., Ahmad I., Scott R.D., 2nd, Foster S.D., Abbasi F., *et al.* (2009).Hospital and societal costs of antimicrobial-resistant infections in a Chicago teaching hospital: implications for antibiotic stewardship. Clin Infect Dis. 49(8):1175-84.
- Robicsek A., Strahilevitz J., Jacoby G.A., *et al*. (2006) Fluoroquinolone-modifying enzyme: a new adaptation of a common aminoglycoside acetyltransferase. Nat Med 12: 83–88. 55.
- Roope, L. S., Smith, R. D., Pouwels, K. B., Buchanan, J., Abel, L., Eibich, P., ... & Wordsworth, S. (2019). The challenge of antimicrobial resistance: what economics can contribute. *Science*, *364*(6435), eaau4679.
- Rosselló, G. A. M., & Pérez, M. Á. B. (2016). Antibiograma rápido en microbiología clínica. *Enfermedades Infecciosas y Microbiología Clínica*, *34*(1), 61-68.

- Russell A. D. (2004). Types of antibiotics and synthetic antimicrobial agents. In: Denyer S. P., Hodges N. A. & German S. P. (eds.) Hugo and Russell‟s pharmaceutical microbiology. 7th Ed. Blackwell Science, UK. Pp. 152-186.
- Rynkiewicz D., Rathkopf M., Sim I., Waytes A.T., Hopkins R.J., Giri L., Nielsen C.J. (2016).Marked enhancement of the immune response to BioThrax® (Anthrax Vaccine Adsorbed) by the TLR9 agonist CPG 7909 in healthy volunteers. *Vaccine.* ; 29(37):6313–6320.
- Sakoulas, G., Bayer, A. S., Pogliano, J., Tsuji, B. T., Yang, S. J., Mishra, N. N., ... & Moise, P. A. (2012). Ampicillin enhances daptomycin-and cationic host defense peptide-mediated killing of ampicillin-and vancomycin-resistant Enterococcus faecium. *Antimicrobial agents and chemotherapy*, *56*(2), 838-844.
- Sakoulas, G., Kumaraswamy, M., Kousha, A., & Nizet, V. (2017). Interaction of antibiotics with innate host defense factors against Salmonella enterica serotype newport. *MSphere*, *2*(6), e00410-17.
- Sakoulas, G., Nonejuie, P., Kullar, R., Pogliano, J., Rybak, M. J., & Nizet, V. (2015). Examining the use of ceftaroline in the treatment of Streptococcus pneumoniae meningitis with reference to human cathelicidin LL-37. *Antimicrobial Agents and Chemotherapy*, *59*(4), 2428-2431.
- Sakoulas, G., Okumura, C. Y., Thienphrapa, W., Olson, J., Nonejuie, P., Dam, Q., ... & Nizet, V. (2014). Nafcillin enhances innate immune-mediated killing of methicillin-resistant Staphylococcus aureus. *Journal of molecular medicine*, *92*, 139-149.
- Sakoulas, G., Rose, W., Berti, A., Olson, J., Munguia, J., Nonejuie, P., ... & Nizet, V. (2017). Classical β-lactamase inhibitors potentiate the activity of daptomycin against methicillin-resistant Staphylococcus aureus and colistin against Acinetobacter baumannii. *Antimicrobial agents and chemotherapy*, *61*(2), e01745-16.
- Sanchez A. R., Rogers R. S. & Sheridan P. J. (2004). Tetracycline and other tetracycline-derivative staining of the teeth and oral cavity. Int. J. Dermatol. 43(10):709-715.
- Santiso R., Tamayo M., Gosalvez J., Bou G., Fernandez M.C., Fernandez J.L. (2011). A rapid in situ procedure for determination of bacterial susceptibility or resistance to antibiotics that inhibit peptidoglycan biosynthesis. BMC Microbiol.11:191.
- Schjørring, S., & Krogfelt, K. A. (2011). Assessment of bacterial antibiotic resistance transfer in the gut. *International journal of microbiology*, *2011*.
- Schlegel H. G. (2003). General microbiology. 7th Ed. Cambridge University Press, Cambridge.
- Sender, R., Fuchs, S., & Milo, R. (2016). Revised estimates for the number of human and bacteria cells in the body. PLoS Biology, 14, e1002533. https://doi.org/10.1371/ journal.pbio.1002533.
- Siegel J.D., Rhinehart E., Jackson M., Chiarello L. (2006). Management of multidrug-resistant organisms in health care settings,. Am J Infect Control 2007 35 (suppl 2): 165-193.
- Sihvonen, R., Siira, L., Toropainen, M., Kuusela, P. & Patari-Sampo, A. (2017). Streptococcus pneumoniae antimicrobial resistance decreased in the Helsinki Metropolitan Area after routine 10-valent pneumococcal conjugate vaccination of infants in Finland. Eur. J. Clin. Microbiol. Infect. Dis. 36, 2109–2116.

- Silva, Diego Romário, Rosalen, Pedro Luiz, Freires, Irlan Almeida, Sardi, Janaína de Cássia Orlandi, Lima, Rennaly Freitas, Lazarini, Josy Goldoni, Lopes da Costa, Tereza Karla Vieira, Pereira, Jozinete Vieira, Godoy, Gustavo Pina, & de Brito Costa, Edja Maria Melo (2019). Anadenanthera Colubrina vell Brenan: anti-Candida and antibiofilm activities, toxicity and therapeutical action. Brazilian Oral Research, 33(23), 1–11. https://doi.org/10.1590/1807-3107bor-2019.vol33.0023 (In press).
- Sköld O. (2011).Antibiotics and Antibiotic Resistance. Hoboken, New Jersey: Wiley & Sons.
- Slatore C. G. & Tilles S. A. (2004). Sulfonamide hypersensitivity. Immunol. Allergy Clin. North Am. 24(3):477-490.
- Soccol, C. R., Prado, M. R. M., Garcia, L. M. B., Rodrigues, C., Medeiros, A. B. P., & Thomaz-Soccol, V. (2015). Current developments in probiotics. Journal of Microbial & Biochemical Technology, 07, 11–20.
- Soto S.M. (2013) Role of efflux pumps in the antibiotic resistance of bacteria embedded in a biofilm. Virulence 4: 223–229.
- St. Louis (2000).Drug facts and comparisons.1280–93.
- Stawinski J., Szafranski K., Vullo D. & Supuran C. T. (2013). Carbonic anhydrase inhibitors. Synthesis of heterocyclic 4-substituted pyridine3-sulfonamide derivatives and their inhibition of the human cytosolic isozymes I and II and transmembrane tumor- associated isozymes IX and XII. Eur. J. Med. Chem. 69:701-710.
- Stefani S., Campanile F., Santagati M., *et al*. (2015) Insights and clinical perspectives of daptomycin resistance in Staphylococcus aureus: a review of the available evidence. Int J Antimicrob Agents 46: 278–289.
- Stuart J.C., Voets G., Scharringa J., Fluit A.C., Leverstein-van Hall M.A. (2012).Detection of carbapenemase-producing Enterobacteriaceae with a commercial DNA microarray. J Med Microbiol. 61:809–12.
- Szajewska, H., Horvath, A., & Piwowarczyk, A. (2010). Meta-analysis: the effects of Saccharomyces boulardii supplementation on Helicobacter pylori eradication rates and side effects during treatment. *Alimentary pharmacology & therapeutics*, *32*(9), 1069-1079.
- Talaro K. P. & Chess B. (2008). Foundations in microbiology. 8th Ed. McGraw Hill, New York.
- Tan T.L., Gomez M.M., Kheir M.M., Maltenfort M.G., Chen A.F. (2017) Should Preoperative Antibiotics Be Tailored According to Patient's Comorbidities and Susceptibility to Organisms? J Arthroplasty. 32(4):1089-1094.e3.
- Tanabe M., Szakonyi G., Brown K.A., *et al.* (2009). The multidrug resistance efflux complex, EmrAB from Escherichia coli forms a dimer in vitro. Biochem Bioph Res Co 380: 338–342.
- Tannock, G. W. (2003). Probiotics: time for a dose of realism. *Current issues in intestinal Microbiology*, *4*(2), 33-42.
- Tarchini G., Liau K.H., Solomkin J.S. (2017). Antimicrobial Stewardship in Surgery: Challenges and Opportunities. Clin Infect Dis. 15; 64(suppl_2):S112-S114.

- Tedijanto C., Olesen S.W., Grad Y.H., Lipsitch M. (2018) Estimating the proportion of bystander selection for antibiotic resistance among potentially pathogenic bacterial flora. *Proc. Natl. Acad. Sci.*; 115(51):E11988–E11995.
- Tekle Y. I. *et al.* (2012). Controlling antimicrobial resistance through targeted, vaccine-induced replacement of strains. PLoS ONE 7, e50688.
- Tenover F.C., Hugles J.M. (1996).The challenges of emerging infectious diseases development and spread of multiply resistant bacterial pathogens. JAMA.
- Ternent L., Dyson R.J. and Krachler A-M. *et al* (2014). Bacterial fitness shapes the population dynamics of antibiotic resistant and susceptible bacteria in a model. J Theor Biol 372:1–11.
- The American Society of Health-System Pharmacists. Archived from the original on 28 December 2016. Retrieved 8 December 2016.
- Thiolas A., Bornet C., Davin-Régli A., *et al.* (2004).Resistance to imipenem, cefepime, and cefpirome associated with mutation in Omp36 osmoporin of *Enterobacter aerogenes*. *Biochem Bioph Res Co.*; 317:851–856.
- Tortora G.J., Funke B.R., Case C.L. (2015).Microbiology: An Introduction, Global Edition. 12th ed. London: Pearson Education.
- Tung, J. M., Dolovich, L. R., & Lee, C. H. (2009). Prevention of Clostridium difficile infection with Saccharomyces boulardii: a systematic review. *Canadian Journal of Gastroenterology*, *23*(12), 817-821.
- Ubeda, C., & Pamer, E. G. (2012). Antibiotics, microbiota, and immune defense. Trends in Immunology, 33, 459–466.
- Uddin B.M.M. (2016). In vitro and in vivo evaluation of antibiotic combination against imipenem resistant Acinetobacter baumannii isolated from patients of DMCH. Department of Microbiology, Dhaka Medical College, January [M.Phil thesis]).
- Ulloa, E. R., Dillon, N., Tsunemoto, H., Pogliano, J., Sakoulas, G., & Nizet, V. (2019). Avibactam sensitizes carbapenem-resistant NDM-1–producing Klebsiella pneumoniae to innate immune clearance. *The Journal of Infectious Diseases*, *220*(3), 484-493.
- V. M. Varagić, M. P. (2009).Milošević, Farmakologija, Elitmedica, Beograd, p. 622-627.
- Van Acker H., Van Dijck P., Coenye T. (2014) Molecular mechanisms of antimicrobial tolerance and resistance in bacterial and fungal biofilms. Trends Microbiol 22: 326–333.
- Van Hoek A. H. A. M., Mevius D., Guerra B., Mullany P., Roberts A. P. & Aarts H. J. M. (2011). Acquired antibiotic resistance genes: An overview. Front. Microbiol. 2:203 doi: 10.3389/fmicb.2011.00203.
- Vázquez-Laslop N., Mankin A.S. (2018).How Macrolide Antibiotics Work. Trends Biochem Sci. 43(9):668-684.
- Vedantam G., Guay G.G., Austria N.E., *et al.* (1998) Characterization of mutations contributing to sulfathiazole resistance in Escherichia coli. Antimicrob Agents Ch 42: 88–93.
- Vikas Manchanda, Sinha Sanchaita, N.P. Singh. (2010).Multidrug Resistant Acinetobacter. J Glob Infect Dis; 2:291-304.
- Villagra N.A., Fuentes J.A., Jofré M.R., *et al*. (2012).The carbon source influences the efflux pump mediated antimicrobial resistance in clinically important Gram-negative bacteria. J Antimicrob Chemoth 67: 921–927.

- Walsh C. (2003). Antibiotics: actions, origins, resistance. 1st Ed. ASM Press, Washington, DC. 345p
- W-Dahl A., Robertsson O., Stefánsdóttir A., Gustafson P., Lidgren L. (2011)Timing of preoperative antibiotics for knee arthroplasties: Improving the routines in Sweden. Patient Saf Surg.19; 5:22.
- Wilson W., Taubert K.A., Gewitz M., et al. (2008). Prevention of infective endocarditis: guidelines from the American Heart Association: a guideline from the American Heart Association Rheumatic Fever, Endocarditis and Kawasaki Disease Committee, Council on Cardiovascular Disease in the Young, and the Council on Clinical Cardiology, Council on Cardiovascular Surgery and Anesthesia, and the Quality of Care and Outcomes Research Interdisciplinary Working Group. JADA. 139 (suppl):3S-24S.
- Wolvers, D., Antoine, J. M., Myllyluoma, E., Schrezenmeir, J., Szajewska, H., & Rijkers, G. T. (2010). Guidance for substantiating the evidence for beneficial effects of probiotics: prevention and management of infections by probiotics. *The Journal of nutrition*, *140*(3), 698S-712S.
- Xu F., Xu H., Wang X., Zhang L., Wen Q., Zhang Y. & Xu W. (2014). Discovery of N-(3-(7H-purin-6-yl)thio)-4-hydroxynaphthalen-1-yl)- sulfonamide derivatives as novel protein kinase and angiogenesis inhibitors for the treatment of cancer: synthesis and biological evaluation. Part III. Bioorg. Med. Chem. 22(4):1487-1495.
- Yang S.J., Kreiswirth B.N., Sakoulas G., *et al.* (2009) Enhanced expression of dltABCD is associated with development of daptomycin nonsusceptibility in a clinical endocarditis isolate of Staphylococcus aureus. J Infect Dis 200: 1916–1920.
- Yang, H., Sun, Y., Cai, R., Chen, Y., & Gu, B. (2019). The impact of dietary fiber and probiotics in infectious diseases. Microbial Pathogenesis, 103931.
- Yoneyama H., Katsumata R. (2006).Antibiotic resistance in bacteria and its future for novel antibiotic development. Biosci Biotechnol Biochem; 70:1060-75.
- Young, V. B., & Schmidt, T. M. (2004). Antibiotic-associated diarrhea accompanied by large-scale alterations in the composition of the fecal microbiota. *Journal of clinical microbiology*, *42*(3), 1203-1206.
- Yüce A. (2001).Antimikrobiyal ilaçlara direnç kazanma mekaniz-maları. Klimik Dergisi.
- Zessel, K., Mohring, S., Hamscher, G., Kietzmann, M., & Stahl, J. (2014). Biocompatibility and antibacterial activity of photolytic products of sulfonamides. *Chemosphere*, *100*, 167-17.
- Zhang Y. (2009) Mechanisms of drug resistance in Mycobacterium tuberculosis. Int J Tuberc Lung Dis 13(11):1320–1330.

المضادات الحياتية:

مصطلح مضاد حيوي تم صياغته من كلمة "antibiosis" والتي تعني حرفياً "ضد الحياة". في الماضي ، كانت المضادات الحيوية تعتبر مركبات عضوية ينتج عن كائن حي دقيق واحد سام للكائنات الحية الدقيقة الأخرى. نتيجة لهذه الفكرة ، تم تعريف المضاد الحيوي في الأصل على نطاق واسع على أنه مادة ينتجها كائن حي دقيق واحد البيولوجية الأصل والذي بتركيزات منخفضة يمكن أن يمنع نمو أو يمنع مميتة للكائنات الدقيقة الأخرى. ومع ذلك ، كان هذا التعريف المعدل في العصر الحديث ، لتشمل مضادات الميكروبات التي يتم إنتاجها أيضًا جزئيًا أو كليا من خلال الوسائل الاصطناعية. في حين أن بعض المضادات الحيوية قادرة على القتل تمامًا بكتيريا أخرى ، بعضها قادر فقط على تثبيط نموها. تلك التي تقتل البكتيريا هي يطلق عليه اسم مبيد للجراثيم في حين أن تلك التي تمنع نمو البكتيريا تسمى مثبطة للجراثيم. على الرغم من أن المضاد الحيوي يشير عمومًا إلى مضاد للجراثيم ، مضاد حيوي يتم تمييز المركبات كمضادات للبكتيريا ومضادات الفطريات ومضادات الفيروسات لتعكس مجموعة من الكائنات الحية الدقيقة التي يتم قتلها.

برزت المضادات الحيوية في جميع أنحاء العالم مع إدخال البنسلين في 1941منذ ذلك الحين قاموا بإحداث ثورة في علاج الالتهابات البكتيرية في البشر والحيوانات الأخرى. ومع ذلك ، فهي غير فعالة ضد الفيروسات.

جمهورية العراق
وزارة التعليم العالي والبحث العلمي
جامعة بابل
كلية العلوم للبنات

لمحة عامة عن مقاومة مضادات الميكروبات:

التصنيف ، طرق العمل والآليات

بحث تخرج مُقدم الى كلية العلوم للبنات ـ جامعة بابل

كجزء من متطلبات نيل شهادة البكالوريوس في قسم علوم الحياة

من قِبل:

زهراء موسىٰ كاظم

زهراء هادي ونّاس

إشراف:

أ.د.حسنين خليل ابراهيم

٢٠٢٣م **١٤٤٤هـ**