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Review on mechanisms of action and resistance of antibiotics

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Abstract
Antibiotics are the substances that can be produced or developed by the microorganism, which selectively inhibit the generation (bacteriostatic) or kill (bactericida the microorganism (that are harmful for human and animal health) at very low concentrations. The mechanism of action of antibiotics can be categorized based on the function that is affected by the agents, these generally included the following: inhibition of the cell wall synthesis, inhibition of the nucleic acid synthesis, inhibition of ribosome function, cell membrane function and inhibition of folate metabolism. 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. 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. Resistance is the ability of a bacteria against the antagonizing effect of an antibacterial agent upon reproduction prevention or bactericidal. The developments of resistance to antibiotics in bacteria often develop as a result of unnecessary and inappropriate use of antibiotics. Several factors have been reported to be responsible to antibiotics resistance in bacterial. Some of the reasons includes: Reduced access to target due to slow porin channels; increased antibiotics expulsion due to multiple drug efflux pumps; inactivating enzymes due to B - lactamases, aminoglycoside-modifying enzymes; mutational resistance due to regulatory mutations that increases the expression of intrinsic genes and operons which is variable in certain circumstances.

Keywords: Antibiotics, classification, mechanism of action, resistance

1. Introduction
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 [14]. With related to this, these (Antibiotics) are the substances that can be produced or developed by the microorganism, which selectively inhibit the generation of or kill the microorganism (that are harmful for human and animal heath) at very low concentrations [46]. While 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 [55].
The mechanism of action of antibiotics can be categorized based on the function that is affected by the agents, these generally included the following: inhibition of the cell wall synthesis, inhibition of the nucleic acid synthesis, inhibition of ribosome function, cell membrane function and inhibition of folate metabolism [14]. antibiotics are one of the most successful forms of therapy in medicine, however the efficiency of antimicrobials is compromised by a growing number of antibiotic resistant pathogens. Increasing rates of bacterial resistance among common pathogens and serious ones are threatening the effectiveness of even the most reliable potent antibiotics. With the ever-increasing spread of multidrug resistance pathogens in our daily lives it becomes imperative to find a way out to suppress this menace because sooner or later the spread will eventually become a serious public concern. Increasing prevalence of resistance has been reported in many pathogens over the years in different regions of the world including developing countries [9].

This has been attributed to changing microbial characteristics, selective pressures of antimicrobial use, and societal and technological changes that enhance the development and transmission of drug-resistant organisms. Although antimicrobial resistance is a natural biological phenomenon, it often enhanced as a consequence of infectious agents' adaptation to exposure to antimicrobials used in humans or agriculture and the widespread use of disinfectants at the farm and the household levels [55]. Therefore, the objective of this paper is to review the classification, mechanism of action and resistance of antibiotics and control strategies of their resistance.

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. 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 [18].

Beta-lactams

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. The most prominent representatives of the beta-lactam class include Penicillins, Cephalosporins, Monobactams and Carbapenems [25].

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 [37]. 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 [37]. 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.

Tetracyclines

Tetracycline was discovered in 1945 from a soil bacterium of the genus *Streptomyces* by Benjamin Duggar [47]. The first member of this class was chlortetracycline (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, Meclocycline, 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 [20].

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 not recommended for young patients because the drugs have shown to cause teeth discoloration [47].

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 [12]. 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 hither to 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 [12]. 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 [12]. 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.

Aminoglycosides

The first drug to be discovered among members of this class of antibiotics was streptomycin, first isolated in 1943 [34]. 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 glyosidic bonds. They are obtained from soil *Actinomycetes*. 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 [42], 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 [51]. 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 Gram-negative rods (*Escherichia*, *Pseudomonas*, *Shigella* and *Salmonella*). Tobramycin, in particular, is used in treating *Pseudomonas* infections in cystic fibrosis patients [22].

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. 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. Studies have shown that Sulphonamides are also able to impede cancerous cell agents [50]. The original antibacterial sulphonamide (also spelt sulfonamide by some Workers), are synthetic antimicrobial agents that contain the sulphonamide group [26].

Sulphonamides are generally thought to be bacteriostatic rather than bactericidal. However, [26] 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 unfavorable to bacteria. Such unfavorable 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 [11].

Glycopeptides

Glycopeptide antibiotics generally abbreviated as GPAs were originally obtained as natural products, but the last 20 years witnessed the emergence of semi-synthetic derivatives with improved activity and pharmacokinetic properties [30, 53]. Naturally, glycopeptides are made of a cyclic peptide of 7 amino acids, to which are bound 2 sugars, hence the name glycopeptides [31]. Binding of the antibiotic to its target occurs via the formation of 5 hydrogen bonds with the peptidic backbone of the drug. Sometimes, an additional chlorine and/or sugar is/are attached to the backbone of the drug (as is the case in oritavancin) during synthesis. Drugs with such additional attachments are known to bind more efficiency to the target [2, 4]. Similarly, a lipophilic side chain antibacterial potency and prolongs half-life of glycopeptides.

Oxazolidinones

Oxazolidinones are a group of synthetic antibiotics approved only recently for use. Linezolid which represents the first member to be synthesized was approved for clinical application only in the year 2000. Although the mechanism of action of oxazolidinone is not yet fully understood, they are reported to interfere with protein synthesis. Oxazolidinones inhibit protein synthesis by binding to the P site of the ribosomal 50S subunit [7]. They have a broad spectrum of activity against Gram-positive bacteria including methicillin- and vancomycin-resistant staphylococci, vancomycin-resistant enterococci, penicillin-resistant pneumococci and anaerobes [7].

Linezolid is used for treatment of respiratory tract and skin infections caused by Gram-positive bacterial pathogens [36]. Oxazolidinones constitute the choice drug in dealing with surgical infections because they easily penetrate and accumulate in the tissue including bone, lung, vegetations (plant-like growth in tissues), haematoma and cerebrospinal fluid. Although adhering to normal standard routines of linezolid administration are usually safe, side effects such as myelosuppression, resulting to anemia and thrombocytopenia are often encountered in cases when treatment is prolonged [7].

Mechanisms of action

Generally, antibiotics act by killing bacteria (bactericidal) or inhibiting their growth (bacteriostatic). Typical examples of bacteriostatic antibiotics are chloramphenicol, tetracyclines or macrolides, while ß-lactam antibiotics, fluoroquinolones or nitrofurantoin are bactericidal antibiotics. To enforce their inhibitory effects, antibiotics need to disturb central cellular processes, without exerting harmful effects to the patient. This can for example be achieved by inhibiting an enzyme or pathway that is essential in bacteria, but not in eukaryotic cells. Alternatively, antibiotics can inhibit targets that diverged from their homologues in eukaryotes to such an extent that a specific binding can be achieved to the bacterial variant alone. The mechanism of action of antimicrobial agents can be categorized based on the function that is affected by the agents, these generally included the following: - Inhibition of the cell wall synthesis, Inhibition nucleic acid synthesis, Inhibition of ribosome function (Protein synthesis), Disrupted cell membrane function and Inhibition of folate metabolism [51].

Inhibition of Cell Wall Synthesis

Most bacterial cells are encased by a rigid layer of peptidoglycan (PG), also called murein in older sources) which both protect the cells in the face of prevailing osmotic pressure consistent with the often-harsh environment and conditions under which they exist. Peptidoglycan has a degree of cross-linking peptide bonds called β-(1-4) –N– acetyl Hexosamine [8],[27]. To stay alive, bacteria must of necessity synthesize peptidoglycan; they do this by the activity of PBPs which are transglucosylases and transpeptidases. These two enzymes play very pivotal roles by adding disaccharide pentapeptides to extend the glycan strands of existing peptidoglycan molecule and also cross-link strands of immature peptidoglycan units [40]. Drugs like penicillin's, carbapenems and cephalosporins are able to block the cross-

linking of peptidoglycan units by inhibiting the peptide bond formation catalyzed by PBPs [29].

Most antibiotics belonging to the glycopeptide class of antibiotics (for example, vancomycin) are able to inhibit bacterial growth by inhibiting the synthesis of PG. They inhibit the synthesis of PG by binding themselves to PG units, as well as blocking transglycosylase and transpeptidase activity [30].

Breakdown of the Cell Membrane Structure or Function

The classes of antibiotics that damage cell membranes of bacteria are specific in each microbial group based on the differences in the types of lipids in their cell membranes. For example, Daptomycin depolarizes calcium-dependent membrane, and that leads to the cessation of macromolecular synthesis and disruption of the cellular membrane in bacteria [1]. The polymyxins cause disintegration of bacterial cell membrane by effectively binding to the lipid moiety of the lipopolysaccharide in the bacterial cell [19].

Inhibition of Nuclei Acid Synthesis

The metabolic pathways that result in synthesis of nucleic acids are very essential; disruption of nucleic acid synthesis is inimical to both the survival and posterity of bacterial cells. Antibiotics interfere with nuclei acid synthesis by blocking replication or stopping transcription. DNA replication involves the unwinding of the traditional double helix structure, a process facilitated by the helicase enzymes [21]. The quinolones group of antibiotics, for example, do interfere with the functionality of the helicase enzyme thereby disrupts the enzyme from playing its function of unwinding DNA. This antibiotic action of the quinolones ultimately truncates the process of DNA replication and repair amongst susceptible bacteria [10]. Antibiotics whose mode of action is inhibition of nucleic acid synthesis also target topoisomerase II and topoisomerase IV of bacteria. Disrupting the activities of these enzymes in bacteria adversely affects RNA polymerase which in turn prevents RNA synthesis. Quinolones that inhibit bacterial nucleic acid synthesis in this way do not interact with mammalian RNA polymerase, making them specifically antagonistic to Gram-positive bacteria and some Gram-negative bacteria.

Inhibition of Protein Synthesis

Living things including bacteria are defined by the amount and type of proteins they are composed of, and continually produce. Proteins are responsible for the structural composition, metabolic and physiological processes, and response to adverse conditions, amongst other roles. However, the type and amount of proteins produced by a bacterium at any given time is dependent on information contained in yet another very important biomolecule – Deoxyribonucleic acid (DNA). DNA determines the type of protein a bacterial cell produces through certain information it harbors within itself. The information is a set of genetic codes called codons, handed down to an identical biomolecule – Ribonucleic acid (RNA), specifically messenger RNA (mRNA). Transfer RNA (tRNA), a similar biomolecule is also formed under the directive of DNA. This biomolecule together with mRNA travels to the ribosomes – the factory for protein synthesis in a living cell. The tRNA then deciphers the codons contained in the mRNA and facilitates the translation of the sequence of codons to a sequence of amino acids which are the building blocks of proteins [17]. The translation of mRNA into proteins occurs over three sequential phases (initiation, elongation and termination) involving the ribosome and a host of cytoplasmic accessory factors [24]. Ribosomes are made up of RNA and proteins, and are generally called ribonucleoproteins. The RNA component is what is referred to as Ribosomal RNA (rRNA), and comprises two subunits, one small subunit (SSU) and the other large subunit (LSU). These two subunits are usually described in terms of their sedimentation coefficients (that is, their rate of sedimentation is an ultracentrifuge), and are measured in Svedberg units (symbols) termed the 30S and 50S, respectively [39].

Bacterial possess 5S, 16S and 23S genes on their rRNA (Moore, 2001). The 16S rRNA gene resides as a single RNA gene in their SSU (16S) whilst the other two rRNA genes (23S and 5S) occur on the LSU of the bacterial ribosome [33]. There is huge difference between prokaryotic and eukaryotic rRNA, and this feat has greatly enabled Scientists to develop antibiotics that would target rRNA of a wide spectrum of pathogenic bacteria [28]. Given the importance of proteins in the metabolic and life processes of all living organisms, whatever disrupts the process of its synthesis in a bacterial cell would ultimately incapacitate the cell; inhibit its growth or even kill it completely. Drugs that inhibit protein synthesis are among the broadest classes of antibiotics and can be divided into two subclasses: the 50S inhibitors and 30S inhibitors. Antibiotics such as erythromycin, clindamycin, lincomycin, chloramphenicol, linezolid etc. have been shown to be among the 50S ribosome inhibitors. In general terms, antibiotics that inhibit 50S ribosome do so by physically blocking either the initiation phase of protein translation or the elongation phase of protein synthesis where the incoming amino acid is linked up with the growing nascent peptide chain. Examples of antibiotic that block initiation of protein translation are members of Oxazolidinones [41] whilst macrolides such as lincosamide and streptogramin block protein synthesis by inhibiting the elongation phase of mRNA translation. These latter groups of antibiotics are therefore reportedly ineffective when elongation has progressed beyond a critical length [52]. The 30S ribosome-inhibitors principally work by blocking the access of aminoacyl-tRNAs to the ribosome. Examples of antibiotics that function in this manner include the tetracycline, streptomycin, spectinomycin [28]. It is worthy to note that some earlier works have shown that tetracycline also inhibits some proteins at the 50S ribosomes. Among ribosome inhibitors, the naturally-derived aminoglycoside subclass is the only one that is broadly bactericidal. Macrolides, streptogramins, spectinomycin, tetracyclines and chloramphenicol are typically bacteriostatic. However, some of these ribosome inhibitory antibiotics that are typically bacteriostatic in action could be bactericidal under certain conditions relating to species- or treatment-specific fashion. For example, chloramphenicol known typically to be bacteriostatic has been shown to effectively kill S. pneumoniae and Neisseria meningitidis [44], as well as H. influenza [23]. This species pecific variability in ribosome inhibition or mediated cell death is potentially linked to sequence differences among bacterial species in the variable regions of the highly conserved ribosomal proteins and RNAs [38].

Blockage of Key Metabolic Pathways

Some antibiotics like sulphonamides and trimethoprim have been shown to mimic a substrate needed for cellular metabolism of bacteria. This deception causes bacterial enzymes to attach themselves to the antibiotic rather than the

normal substrate. In particular, sulphonamides act like tetrahydrofolate which is required for the synthesis of folic acid in bacterial cells. Folic acid is vital in the metabolism of nucleic acid and amino acids; for this reason, sulphonamides ultimately disrupt the production of nucleic acids (DNA and RNA) and amino acids, as they mimic substrates required for folic acid metabolism [51].

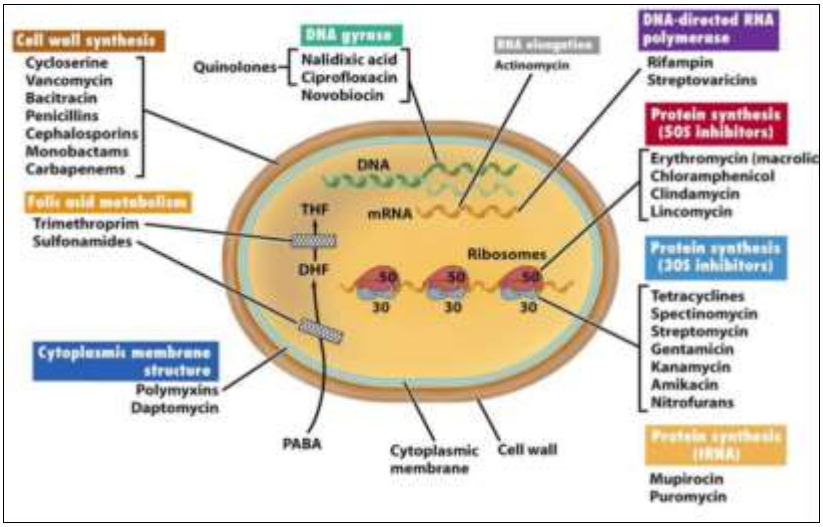

Fig 1: Short summery antibiotic target sites and their mechanism of action [18].

Mechanism of resistance

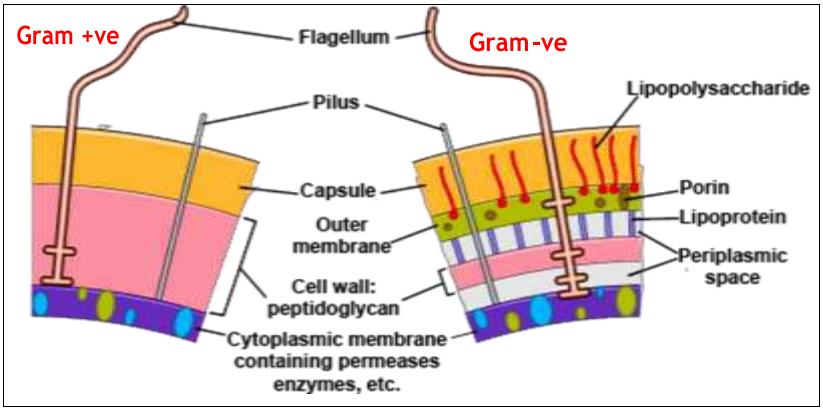

Fig 2: Cell structure of gram positive and gram negative bacterial

Antimicrobials are one of the most successful forms of therapy in medicine; however, the efficiency of antimicrobials is compromised by a growing number of antibiotic resistant pathogens. Several factors have been reported to be responsible to antibiotics resistance in bacterial. Some of the reasons includes: Reduced access to target due to slow porin channels; increased antibiotics expulsion due to multiple drug efflux pumps; inactivating enzymes due to B - lactamases, aminoglycoside-modifying enzymes; mutational resistance due to regulatory mutations that increases the expression of intrinsic genes and operons which is variable in certain circumstances [38]. 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. Resistance can be described in two ways: Natural (Intrinsic) resistance, acquired resistance. Intrinsic resistance whereby microorganisms naturally do not posses target sites for the antimicrobials and the antimicrobial does not affect them or possess natural barriers that prevent the agents from reaching the target; examples are the natural resistance of gram- negative bacteria to vancomycin because of the drug's inability to penetrate the gram-negative outer membrane, or the intrinsic resistance of the penicillin [6],[57]. 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. Whereas acquired resistance whereby a naturally susceptible microorganism acquires mechanism to not be affected by the antimicrobial. Mechanisms of acquired resistance include: the presence of an enzyme that inactivates the antimicrobial agent, post- transcriptional or post-translation modification of the antimicrobial agent's target, reduced uptake to the antimicrobial agent and active efflux of the antimicrobial agent. The major resistance mechanisms of microbes are decreased drug uptake, efflux pumps, enzymes that inactivate

an antimicrobial chemical and target *al*terations by mutation and biofilms.

Gram positive bacteria have a cell wall composed mostly of peptidoglycan, a very rigid substance. This is a prime target of β lactam antimicrobials such as penicillin's and cephalosporins. The antimicrobial locks on to the β lactam structure in the cell wall, preventing expansion, and the cell ruptures as it grows. Gram negative bacteria have a much thinner cell wall itself and this is protected by a lipopolysaccharide molecule in the capsule, an outer membrane and what is known as the periplasmic space. In short it is a much more heavily armoured vehicle. Porins are openings in the cytoplasmic membrane through which antimicrobial agents can gain entry a reduced number of such porins is one means of antimicrobial resistance.

Efflux Pump

Some bacteria, e.g. Pseudomonas, have a system called an efflux pump. As its name suggests this is a system whereby the bacterium has a pump to expel ingested chemicals. Although some of these drug efflux pumps transport specific substrates, many are transporters of multiple substrates [49]. Antimicrobial efflux pumps are believed to contribute significantly to acquired bacterial resistance because of the very broad variety of substrates they recognize, their expression in important pathogens, and their cooperation with other mechanisms of resistance, such as decreased uptake. Their presence also explains high-level intrinsic resistances found in specific organisms. The design of specific, potent efflux pump inhibitors appears to be an important goal for the improved control of infectious diseases in the near future. For example, in ear therapy tris-EDTA has the potential to partially inactivate the efflux pump but this is only a topical specified action not generally available in most situations [56].

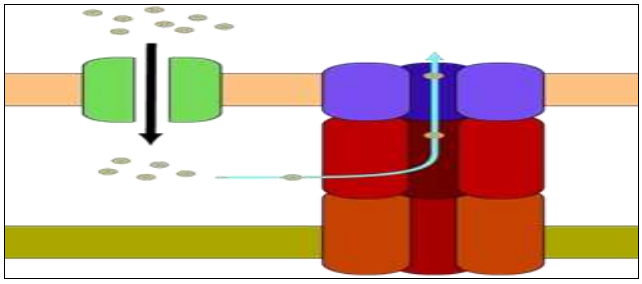

Fig 3: Efflux Pump

Enzyme inactivation

Most of Gram-positive and Gram-negative bacteria synthesize enzymes that degrade antibiotics. This enzymatic inactivation mechanism is one of the most important mechanisms of resistance. In this group, beta-lactamases, aminoglycosides, modifying enzymes (acetylase, fosforiaz adenilaz and enzymes) degrade beta-lactam antibiotics and continually increasing their number of which inactivates enzymes include chloramphenicol and erythromycin [35]. The most notable example is penicillinase that can inactivate penicillin, but there are others. Clavulanic acid can bind penicillinase leaving the antimicrobial amoxicillin to do its work, and also there are the penicillinase resistant penicillin's such as methicillin and cloxacillin, but they are still subject to target *al*terations (see below) making them ineffective over time.

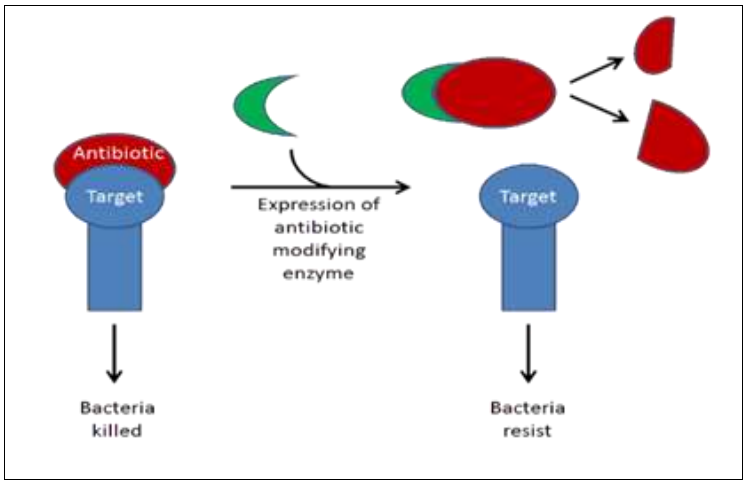

Fig 4: Enzyme inactivation

Mutation

When an antimicrobial attack a specific target, whether it be cell wall peptides, ribosomes or nuclear DNA, it locks on to specific receptors on the target. Bacterial mutation results in the alteration of these receptors so that the antimicrobial can no longer fit and the organism is thus resistant to the effects of the antimicrobial. Examples of clinical strains showing resistance can be found for every class of antimicrobial, regardless of the mechanism of action. Target site changes often result from spontaneous mutation of a bacterial gene on the chromosome and selection in the presence of the antimicrobial. Thus, antimicrobials resistant to penicillinase may still be rendered ineffective. This has led to the term methicillin resistant Staphylococcus aureus (MRSA) are the typical multi-resistant organism [15].

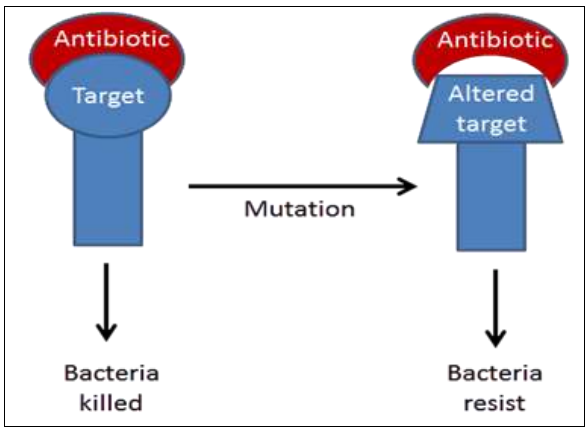

Fig 5: Mutation

Horizontal gene transfer

Horizontal gene transfer (HGT) is a major mechanism for the growth of antimicrobial resistance (AMR). HGT is more rapid than simple mutation. Horizontal gene transfer is genetic modification by microorganisms themselves and is a very efficient and rapid way of transferring resistance between populations. It is the most relevant mode of resistance emergence and spread in microbial populations. The main methods are transformation, transduction and conjugation [5].

Transformation

Transformation refers to the ability of microorganisms to utilise snippets of free DNA from their surroundings. DNA from dead cells gets cut into fragments and exits the cell.

The free-floating DNA can then be picked up by competent cells. Exogenous DNA is taken up into the recipient cell from its surroundings through the cell membrane. The exogenous DNA is incorporated into the host cell's chromosome via recombination. Transformation results in the genetic alteration of the recipient cell.

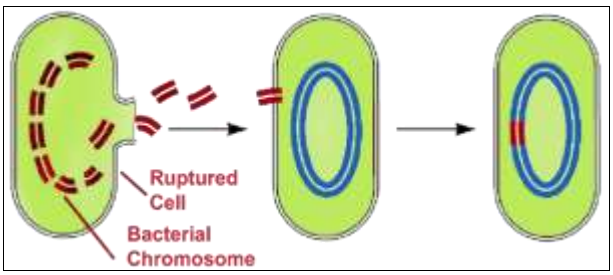

Fig 6: Horizontal gene transfer

Transduction

Transduction is the process by which viruses that prey upon bacteria, known as bacteriophages, can transmit genetic material from one organism to another. It is similar to the way mosquitos transmit disease from animal to animal. However, while the mosquito is a passive carrier, bacteriophages are more complicated. Being viruses themselves they inject their genetic material into a bacterial cell and replicate there to a great degree. Their normal mode of reproduction is to harness the replicational, transcriptional, and translation machinery of the host bacterial cell to make numerous virions, or complete viral particles, including the viral DNA or RNA and the protein coat. The packaging of bacteriophage DNA has low fidelity and small pieces of bacterial DNA, together with the bacteriophage genome, may become packaged into the bacteriophage genome. At the same time, some phage genes are left behind in the bacterial chromosome. When the cell eventually ruptures it emits many more bacteriophages into the surroundings to infect other microorganisms.

Conjugation

Bacterial conjugation is the transfer of genetic material between bacterial cells by direct cell-to-cell contact or by a bridge-like connection between two cells. It is a mechanism of horizontal gene transfer as are transformation and transduction although these two other mechanisms do not involve cell-to-cell contact. Bacterial conjugation is often regarded as the bacterial equivalent of sexual reproduction or mating since it involves the exchange of genetic material. During conjugation the donor cell provides a conjugative or mobilizable genetic element that is most often a plasmid or transposon.

The fact that this process can occur easily between different species of bacteria makes it especially important. The process is as described in the graphic below:

Conjugation diagram 1- Donor cell produces pilus. 2- Pilus attaches to recipient cell and brings the two cells together. 3- The mobile plasmid is nicked and a single strand of DNA is then transferred to the recipient cell. 4- Both cells synthesize a complementary strand to produce a double stranded circular plasmid and also reproduce pili; both cells are now viable donor or F-factor.

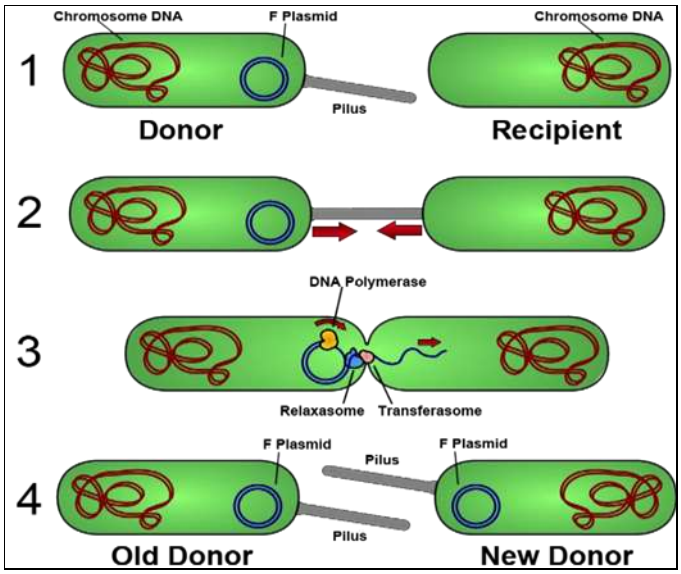

Fig 7: Conjugation

Biofilms

Biofilms are complex microbial communities containing bacteria and fungi. The microorganisms synthesise and secrete a protective matrix that attaches the biofilm firmly to a living or non-living surface. At the most basic level a biofilm can be described as bacteria embedded in a thick, slimy barrier of sugars and proteins. The biofilm barrier protects the microorganisms from external threats. Biofilms have long been known to form on surfaces of medical devices, such as urinary catheters, endotracheal and tympanostomy tubes, orthopaedic and breast implants, contact lenses, intrauterine devices (IUDs) and sutures. They are a major contributor to diseases that are characterized by an underlying bacterial infection and chronic inflammation, e.g. periodontal disease, cystic fibrosis, chronic acne and osteomyelitis. Biofilms are also found in wounds and are suspected to delay healing in some. Planktonic bacteria attach within minutes and form strongly attached micro colonies within 2–4 hours. They become increasingly tolerant to biocides, e.g. antimicrobials, antiseptics and disinfectants, within 6–12 hours and evolve into fully mature biofilm colonies that are extremely resistant to biocides and shed planktonic bacteria within 2–4 days, depending on the species and growth conditions. They rapidly recover from mechanical disruption and reform mature biofilm within 24 hours [13].

A unique property of polymicrobial biofilms is the cooperative protective effects that different species of bacteria can provide to each other. For example, antimicrobial resistant bacteria may secrete protective enzymes or antimicrobial binding proteins that can protect neighbouring non-antimicrobial resistant bacteria in a biofilm, as well as

transfer genes to other bacteria that confer antimicrobial resistance, even between different species [43].

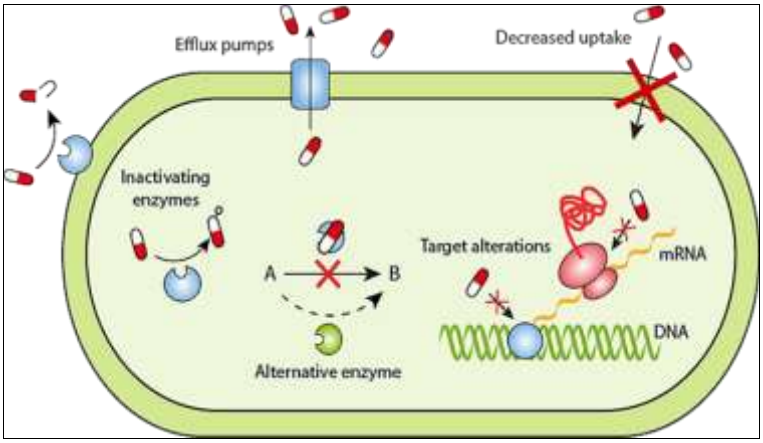

Fig 8: Short summary mechanism of antibiotics resistance

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