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Review on Antimicrobial Resistance: Mechanisms of Spread and Novel Approaches to Diagnostics

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Abstract

Multidrug-resistant (MDR) microorganisms and a dearth of new antibiotics have made antimicrobial resistance an issue for global public health. Through direct contact, the food chain, or pollution of the shared environment, zoonotic diseases and resistance bacteria can spread from animals to people. Genes that provide resistance can be horizontally transferred between bacteria through conjugation, transduction, or transformation. As there are numerous ways that antibiotics can prevent bacterial growth and reproduction or kill existing bacteria, there are numerous ways that bacteria can become resistant to antibiotics. Currently, various methods are employed to detecte antimicrobial resstance(AMR) genes.These includes includes polymerase chain reaction (PCR), quantitative real time PCR (RT-PCR), multiplex PCR, whole genome sequencing(WGS), DNA microarray, metagenomics and Matrix- assisted laser desorption ionization- time of flight mass spectrometry (MALDI-TOF-MS) arenew methods in future (AMR) characterization. Ethiopian government authorities are not well-versed in the ethical use of medications in veterinary care, and they have little control over pharmaceuticals. The two main agencies interested in this issue are the Ministry of Agriculture and Natural Resources and the Ministry of Health.The emergence, prevalence, and development of antimicrobial resistance were made possible by drug residue, drug usage, abuse, and overuse.Therefore awareness creation should be conducted on rational use of drugs for the community and other stake holders and research needs to be developed on the best ways to mitigate the development of antimicrobial resistance.

Keywords: Antibiotics, Mechanisms, Zoonosis, Resistance

List of Abbreviation

AMR	Anti-microbial Resistance
CDC	Centers for Disease Control and prevention
DNA	Deoxyribo-Nucleic Acid
FDA	Food and Drug Administration
FQ	Fluoroquinolone
GIT	Gastrointestinal tract
HGT	Horizontal Gene Transfer
MDR	Multi-Drug Resistance
MRSA	Methicillin Resistant Staphylococcus aureus
PBP	Peniciline Binding protein
RNA	Ribo-Nucleic Acid
WHO	World Health Organization

1. Introduction

The emergence of multidrug-resistant (MDR) bacteria and the scarcity of novel antibiotics have made antimicrobial resistance a major worldwide public health problem. Through direct contact, the food chain, or environmental pollution, resistant bacteria, including zoonotic diseases, can spread between people and animals [1]. Antimicrobial resistance (AR) is the ability of a microorganism to resist the effects of antibiotics it was once able to cure. It is arefers to bacteria's ability to endure and proliferate in the presence of previously effective antibiotic dosages. Although the source of antimicrobial resistance genes is unknown, investigations utilizing clinical isolates obtained prior to the introduction of antibiotics showed sensitivity despite the presence of conjugative plasmids. Treatment for resistant bacteria is more challenging and may include using stronger antibiotic dosages The following paper examines from [2,3].

Exposure to a particular antibiotic usually causes most naïve, vulnerable to infection bacteria to become sensitive to it as well. Nonetheless, in the event that antibiotics are insufficient, a small minority of resistant bacteria will always exist. By removing this subpopulation, the environment will be left open to the growth of microorganisms. Reduced usage of antibiotics has been proposed to exert selection pressure for the development of resistance, which will ultimately help the more fit susceptible bacteria outcompete resistant forms [4]. The ability of bacteria to endure, proliferate, and engage in competition within their surroundings is known as bacterial fitness. Antibiotic-resistant bacteria frequently experience a fitness cost, which lowers their capacity to proliferate overall [5]. This trade-off arises from the fact that the acquired resistance mechanisms or genetic alterations that confer antibiotic resistance to bacteria can also obstruct vital biological functions like energy production, nutrition intake, or growth rate [3]. Because non-resistant strains can outcompete resistant ones in situations lacking antibiotics, resistant bacteria may be less competitive in those settings. Thus, whereas antibiotic resistance allows bacteria to thrive when antibiotics are present, it frequently results in decreased fitness in other environments [2]. Antimicrobial resistance is becoming a major worldwide public health problem due to the prevalence of multidrug-resistant (MDR) bacteria and shortage of novel medications [6]. Through direct contact, the food chain, or microbial pollution of the shared environment, animals might potentially transmit resistant germs and zoonotic illnesses to humans [7].

An increasing public health problem is antimicrobial resistance because diseases caused by resistant bacteria are more difficult and expensive to cure. For example, several Salmonella strains have developed resistance to a variety of medicines during the 1990s (refs). Antibiotic usage in human and animal agriculture is thought to be the cause of resistance. The development of multiple-drug resistance, or resistance to various kinds of antimicrobial agents, is the main issue facing clinical practice today [8].

Humans might be impacted by the bacterial resistances that develop inside animals. The health of humans might be at danger from zoonotic disease caused by resistant bacteria. A resistant type of bacteria is more likely to infect workers at farms or facilities that provide food for animals [9]. Effective treatment of both human patients and animals is becoming increasingly difficult due to the alarmingly frequent and widespread occurrence of infections or diseases linked to antibiotic resistance [6].

Antibiotic abuse and misuse in veterinary and medical settings contribute to this developing resistance, which is further made worse by the environmental introduction of antibiotics. Antibiotics produce a selective pressure that encourages the survival and growth of resistant bacteria when they find their way into natural ecosystems through wastewater, agricultural runoff, or inappropriate disposal [5]. The proliferation and dissemination of these resistant bacteria contribute to a cycle that strengthens antibiotic resistance, hence decreasing the efficacy of current treatments and presenting a severe risk to human and animal health [10].

In general, there are worries about certain common infections that are getting harder to treat. An illness caused by bacteria resistant to antibiotics may take longer to heal, and in order to maintain the efficacy of antibiotics, it is crucial to look into how these medications are used in both humans and animals. To address the growing number of diseases brought on by resistant bacteria and to stop the worrying trend of antibiotic resistance, a variety of new efforts are being implemented [11].

Antimicrobial resistance is a global issue that threatens the treatment of common infections in communities, hospitals, animals, and wild life. The rise of antibiotic resistance can result from misuse in humans and animals, leading to increased mortality, morbidity, treatment costs, and loss of production [12]. Inadequate awareness and surveillance on control and prevention of antibiotic resistance are evident. In addition, some bacteria may develop resistance for all antibiotics, highlighting the need for reform in pharmaceutical innovation.

In clinical practice, multidrug resistance is a major issue and a significant concern. Researchers are continuously investigating preventive approaches and developing robust diagnostic techniques to combat this challenge. The (WHO, 2014)'s report emphasizes the importance of reviewing and understanding antimicrobial resistance to mitigate its harmful effects. Reviewing antimicrobial resistance, often referred to as a silent pandemic, is of paramount importance. Compiling scattered information and facts about antimicrobial resistance is crucial to support efforts aimed at mitigating its detrimental effects. Therefore, the objective of this review paper was:-

✓ To review antimicrobial resistance, its mechanisms of development, novel approaches to diagnostics and its public health significance.

2. Literature Review

2.1. Antimicrobial Resistance

A microbe's capacity to fight against the effects of a drug that it

was formerly able to effectively cure is known as antimicrobial resistance, or AMR. As a subset of antimicrobial resistance (AMR), antibiotic resistance (ABR) refers only to the development of antibiotic resistance in bacteria. The term "antibiotic resistance" refers to a bacteria's capacity to endure and proliferate when exposed to antibiotic dosages that were once believed to be effective against it. Although the source of antibiotic resistance genes is unknown, research conducted on clinical isolates obtained prior to the introduction of antibiotics showed sensitivity despite the presence of conjugative plasmids. Treatment for resistant bacteria is more challenging and may include using stronger antibiotic dosages [13].

2.2. Mechanisms of Antimicrobial Agents

Antibiotics are chemicals which work to halt the development of germs either by killing the bacteria (bactericidal action) or by preventing their division (bacteriostatic action). Although they are not identical, the phrases "antibiotic" and "antimicrobial" are frequently used synonymously Antibiotics are compounds originating from microorganisms, like penicillin. The most significant class of antibacterial agent for combating bacterial illnesses is an antimicrobial chemical that is active against bacteria. While the term "antimicrobial" refers to any substance that works against any kind of microorganism, including synthetic substances that kill bacteria, viruses, fungi, and protozoa (antiprotozoal) [14].

The biochemical process via which a medication exerts its pharmacological effects is known as its mode of action. This could be a particular target that the medication attaches to, such as a receptor or an enzyme, as is the case with many antibiotics. The biological procedure is particularly described at the molecular level by the mechanism of action. Targeting the cell wall, which is missing in human (eukaryotic) cells but present in prokaryotic (bacterial) cells, is one of the most popular modes of action. Antimicrobial drugs, therefore, have a limited impact on host activities and preferentially target essential bacteria functions. Various antibiotic classes have distinct mechanisms of action that either stop or illuminated bacterial growth [15].

Groups of Antibiotics	Mechanisms of Action
Chloramphenicol	Inhibitor of protein synthesis
Penicillin's	Inhibitor of cell wall synthesis
Cephalosporin	Inhibitor of cell wall synthesis
Tetracycline	Inhibitor of DNA synthesis
Aminoglycosides	Inhibitor of protein synthesis
Sulfonamides	Competitive inhibitors of folic acid synthesis
Quinolones	Inhibitor of DNA synthesis
Macrolides	Inhibitors of protein synthesis
Source: [16].	

Table 1: Groups of Antimicrobial Agents and their Modes of Action

2.2.1. Inhibitors of Cell Wall Synthesis

The bacterial cell wall, composed of lengthy sugar polymers called peptidoglycan, envelops the cell. Transglycosidases cause the peptidoglycan to undergo cross-linking of the glycan strands, and the peptide chains stretch from the polymer sugars to establish cross linkages between individual peptides. In the presence of penicillin binding proteins (PBPs), glycine residues cross link the D-alanyl-alanine part of the peptide chain. The cell wall is reinforced by this cross-linking. The cell wall production is inhibited by both glycopeptides and β-lactams [17].

The PBPs are the main targets of the β-lactam drugs. The β-lactam ring is thought to resemble the D-alanyl-alanine segment of the peptide chain, which is often bound by PBP. PBP interacts with the β-lactam ring and isn't accessible for peptidoglycan production. The bacteria lyses when the peptidoglycan layer is disrupted [18]. The precursor of the peptidoglycan subunit's D-alanyl-alanine part of the peptide side chain is bound by the glycopeptides. Cell wall production is inhibited by the big medicinal molecule vancomycin, which blocks this D-alanyl subunit from interacting with the PBP [18].

2.2.2. Inhibitors of Protein Biosynthesis

Transcription is the process by which the information contained in bacterial DNA is first utilized to create messenger RNA (m-RNA), an RNA molecule. Next, in a process known as translation, the macromolecule known as the ribosome synthesises the proteins found in m-RNA. Ribosomes and cytoplasmic factors accelerate the production of proteins. Two ribonucleoprotein subunits, the 30S and 50S subunits make up the bacterial 70S ribosome. Antimicrobials work by targeting the 30S or 50S component of the bacterial ribosome to impede the production of proteins [19,20].

2.2.3. Folic acid Metabolism Inhibitors

A vital ingredient required for the production of proteins and nucleic acids (DNA and RNA) is folic acid. It is a water-soluble vitamin that is an effective free-radical scavenger and methyl donor (B9). All cells need folic acid to develop, and it is produced by bacteria from the substrate para-amino-benzoic acid (PABA). As a vitamin found in diets, folic acid diffuses or enters mammalian cells. However, neither active transport nor diffusion will allow folic acid to pass through bacterial cell walls. Bacteria must thus convert PABA into folic acid. As a substrate for the enzyme dihydropteroate synthase, which converts PABA into dihydropteroic acid, the direct precursor

of folic acid, sulfonamides work by competing with PABA? All these medications block different stages of the metabolism of folic acid. Trimethoprim and sulfa medications working at different stages on the same biosynthesis pathway exhibit synergy and a lower risk of resistance mutation when combined. Compared to p-aminobenzoic acid, the enzyme's natural substrate, sulfonamides have a greater affinity for dihydropteroate synthase and block it in a competitive way. Drugs like trimethoprim block the enzyme dihydrofolate reductase and operate later in the production of folic acid [21].

3. Antimicrobial Resistance Mechanisms

Bacteria produce a wide range of resistance mechanism, which is present in them either innately or through exposure to antibiotics developed over time. These mechanisms can either kill or interfere with the growth and proliferation of bacteria. if antibiotics have a similar mechanism of action an organism can easily become resistant to many different antibiotic classes by one simple resistance force If a microbe is "significantly reduced susceptibility" as compared to both an aggregate of susceptible strains or the "original isolate," it's referred to as resistant. Resistance may arise either by acquisition from non-consanguineous genes via horizontal gene transfer or else through mutation in structural or maintenance and control genes [22]. From time to time, individual bacteria can transfer their resistance by creating plasmids which can be "resistance plasmids" fragments of DNA that are able to move from one cell to another [23].

On the other hand, the infecting bacterium could carry a natural resistance to an antibiotic, and on the other it can develop via acquisition. Acquired resistance may occur with the transfer if additional chromosomal genetic material or by mutation. Finally, during treatment, resistant organisms are selected [22]. It is the most fundamental way in which an organism can develop resistance through a genetic mutation. Owing to the huge advantage afforded by their ability to survive, resistant mutants will use antibacterial treatments overall more frequently within a population and produce isolates that are of an increased proportion which are resistant against these agents [24]. Antimicrobial resistance has several causes. The first is reduced antibiotic accumulation due to decreased permeability or increased efflux. Second, target changes can be occur leading to some of the following actions; target replacing, mutations in the site, enzymatic alteration of site, protection and overproduction. Lastly, there may be alterations that arise from antibiotics like damage or modifications thereof. On the other hand, the global adaptability of a bacterial cell can be at fault for antibiotic resistance by a single cellular structure [25].

3.1. Intrinsic or Natural or Passive

Due to the chemical differences between the drug and the microbial membrane structures particularly for those that need entry into the microbial cell to affect their action microorganisms in this case either naturally lack target sites for the drugs, meaning the drug has no effect on them, or they naturally have low permeability to those agents. Pseudomonas aeruginosa is an example of a natural resistance agent; its inherent resistance to several antimicrobials is probably mostly due to its limited membrane permeability. Other instances include the outer membrane of Gram-negative bacteria, the existence of genes that provide resistance to self-produced antibiotics, the lack of an antimicrobial uptake transport system, or the general absence of the target or process hit by the antimicrobial [21].

3.2. Acquire or Active Resistance

The main cause of antimicrobial resistance is a particular evolutionary pressure that leads to the development of a defense mechanism against an antibiotic or class of antibiotics, making bacterial populations that were previously susceptible to anti-microbial resistant. The bacterial genome has changed, resulting in this kind of resistance. In bacteria, resistance may be acquired by mutation and transferred vertically to daughter cells through selection. Resistance is most frequently acquired through horizontal gene transfer across strains and species. Gene exchange can occur by conjugation, transduction, or transformation [26].

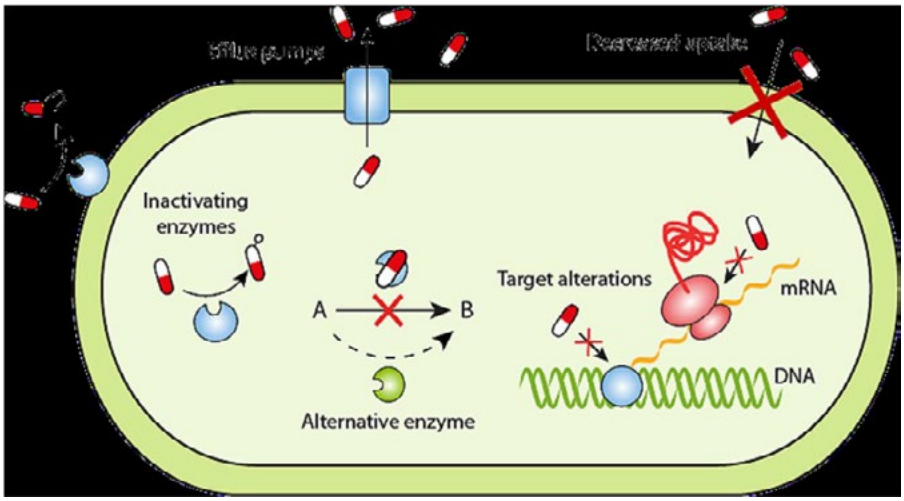

Figure 1: Resistance Mechanism of Bacteria
Source: Adopted from http://www.chembio.uoguelph.ca, sited on July 26, 2023.

3.3. Antibiotics Inactivation

The cell wall of bacteria sometimes develops resistance to antibiotics by producing an enzyme that neutralizes the medication's effects or reduces its effectiveness. Chloramphenicol acetyltransferases, β-lactamases and aminoglycoside-modifying enzymes are the three primary enzymes that render antibiotics inactive. Beta lactamases with ester and amide bonds are the greatest example. The genes those codes for β-lactamases are commonly referred to as bla, and they are located in chromosomes or mobile gene elements (MGEs) that are part of the auxiliary genome blaKPC, for example, is the name of the particular enzyme. When it comes to expression, these genes' transcription may be constitutive or necessitate an outside stimulus to start Penicillin, monobactams, cephalosporin's, and carbapenems are the best examples of beta lactam antibiotics whose beta-lactam rings can be broken by the enzyme β-lactamases. The antibiotic is prevented from attaching to the peptidoglycan precursors in this way by the beta-lactam ring breaking. However, as long as the organism generates beta lactamases, the likelihood of penicillin or other comparable medications being able to compromise the integrity of the cell wall will be reduced [27]. This mode of resistance is prevalent in methicillin-resistant Staphylococcus aureus (MRSA) strains and can be passed from one bacterium to another through the synthesis of plasmids [28].

3.4. Reduced Membrane Permeability

The cytoplasmic membrane, or the inner membrane, is home to many of the antimicrobial agents used in clinical practice when they target gram-negative bacteria or intracellular targets. Bacteria have evolved defense mechanisms that lower the absorption of the antimicrobial molecule, preventing the antibiotic from reaching its intracellular or periplasmic target. Actually, the outer membrane serves as the body's first line of defense against the entry of several harmful substances, including a number of antibacterial drugs. Since hydrophilic compounds, such tetracyclines, fluoroquinolones and β-lactams frequently traverse the outer membrane through water-filled diffusion channels called porins. The permeability of the membrane can have a significant impact. Vancomycin and other glycopeptide antibiotics are ineffective against gram-negative infections due to their inability to cross the outer membrane. Other bacteria Enterobacteriaceae, like Pseudomonas and Acinetobacter baumanii have an intrinsic poor sensitivity to β-lactams, which might be partially attributed to a decreased quantity and/or differential expression of porins [29]. The three main proteins produced by E. Coli (OmpF, OmpC, and PhoE) and P. aeruginosa OprD (also known as protein D2) are among the best-characterized porins and are typical instances of porin-mediated antibiotic resistance. Porins can be altered by three primary methods. (i) changing the kind of porins expressed; (ii) altering the amount of porin expression; and (iii) impairing the function of the porin. Crucially, variations in permeability resulting from any of these pathways typically cause low-level resistance and are commonly linked to other resistance mechanisms, such increased expression of efflux pumps [30].

3.5. Chemical Alteration of Antibiotic

In both gram positive and gram negative bacteria, the development of enzymes that may alter the chemical structure of the antibiotic molecule is a well-known mechanism of acquired antibiotic resistance. There are several kinds of modifying enzymes that have been identified, and the most common biochemical processes that they catalyze are as follows: i) phosphorylation (chloramphenicol, aminoglycosides), ii) acetylation (streptogramins,chloramphenicol, aminoglycosides), and iii) adenylation (lincosamides, aminoglycosides,). Whatever the biochemical process, the end consequence is frequently associated with steric hindrance, which lowers the drug's avidity for its intended target [31]. Aminoglycoside modifying enzymes (AMEs) are key examples of drug resistance by modification, often found in Mobile Genetic Elements (MGEs) or chromosomes containing AME genes in other bacterial species [32]. Chloramphenicol, an antibiotic that inhibits protein synthesis, is primarily altered by acetyltransferases called CATs. These genes are found in both gram-positive and gram-negative bacteria. Type B causes low-level resistance, while type a results in high-level resistance. This enzyme alters the antibiotic's chemical properties [33].

3.6. Changes in Target Sites

Antibiotics bind to specific molecular targets within microbes, but resistance can arise from alterations in these sites. Mutations in bacterial genes can lead to these alterations. Even small changes can significantly impact antibiotic binding. For example, tetracyclines can bind to the transfer RNA access site, preventing antibiotic binding, leading to tetracycline-resistant microorganisms. These resistance mechanisms can be attributed to the precise interactions between antibiotics and target molecules [13]. Antibiotic resistance in bacteria involves a target located in a bacterial chromosome. MGEs carry most of the therapeutically relevant genes implicated in this mode of resistance. Examples include Tetracycline, fluoroquinolones, and fusidic acid. Tetracycline resistance determinants Tet(M) and Tet(O) are prominent examples of this mechanism, involving GTP-dependent interaction with the ribosome [34].

Another strategy of resistance is mutation of the target location. As was briefly described above, the mechanism of FQ(Fluoroquinolone) resistance is one of the most famous examples of mutational resistance. By inhibiting DNA gyrase and topoisomerase IV, two essential enzymes that affect DNA replication, FQs cause bacterial death. Acquired resistance to these drugs most commonly results from chromosomal mutations in the genes encoding subunits of the enzymes indicated above (gyrA-gyrB for DNA gyrase and parC-parE for topoisomerase IV, respectively) [34]. Macrolide resistance is a common issue due to enzymatic modification of the target site, such as methylation of the ribosome by enzymes like erythromycin ribosomal methylation. This alteration hinders the antibacterial molecule's ability to attach to its target, and the expression of erm genes provides cross-resistance to all members of the MLSB group, as macrolides, lincosamides, and streptogramin B antibiotics have overlapping binding sites in the 23S rRNA [35].

Complete replacement or bypass of the target site allows bacteria to evolve new targets with similar biochemical activities without antimicrobial inhibition. Clinical examples include vancomycin resistance in enterococci due to peptidoglycan structure changes and methicillin resistance in S. aureus due to exogenous (PBP2a.) [14]. Gram-positive bacteria resistance is primarily due to changes in penicillin binding protein (PBP), while Gram-negative bacteria resistance is caused by the development of β-lactamases. Streptococcus pneumoniae and Enterococcus faecium resistance is due to the integration of the "staphylococcal cassette chromosome mec" gene into the S. aureus chromosome, resulting in resistance to β-lactam antibiotics [14].

The other mechanisms are modified cell wall precursors are used in the other binding site modification procedures. Glycopeptides such as vancomycin or teicoplanin, when bound to the D-alanyl-D-alanine residues of peptidoglycan precursors, can block the formation of cell walls in Gram-positive bacteria. Resistance to them develops because D-alanyl-alanine is converted to D-alanyl-lactate, which prevents glycopeptides from cross-linking with them. Vapor A-type resistance to vancomycin and teicoplanin is highly prevalent in isolates of Escherichia coli and Escherichia faecalis [14]. DNA gyrase A subunit binds to quinoléne, causing resistance through topoisomerase IV and DNA gyrase. A mutation in gyr A and par C genes prevents replication, preventing FQ attachment [36].

3.7. Efflux or Transport of Antibiotic

The development of sophisticated bacterial machinery that can force a hazardous substance out of the cell can also lead to antibiotic resistance. The use of an efflux pump is one more way that bacteria might develop antibiotic resistance. A biological pump known as an efflux pump has the ability to drive an antibiotic out of the cell, preventing it from reaching or adhering to its target. This mechanism of antibiotic resistance can frequently lead to resistance to multiple classes of antibiotics, particularly the macrolides, tetracyclines, and fluoroquinolones, which inhibit distinct aspects of DNA and protein biosynthesis and thus require intracellular delivery to be effective [37].The five main families of efflux pumps are the ABC, SMR, RND, and MATE. They differ in energy source, substrate variety, structural conformation, and bacterial species. Tetracycline resistance is a well-known example of efflux-mediated resistance, where Tet efflux pumps use proton exchange to extrude tetracyclines, part of the MFS family [16].

3.8. Multiple Antibiotic Resistances

Multiple antibiotic resistances, which can be mediated by the same R-factor, is a phenomenon associated with R-plasmids that contain areas containing resistance genes. Although the occurrence of bacteria with multiple drug resistance is a severe issue in and of itself, clinicians are even more concerned about reducing infections in medical and veterinary practices when multiple drug resistance is transferred to other members of the Enterobacteriaceae family, specifically E. coli, Salmonella, and Shigella [16].

3.8.1. Transfer of Antibiotic Resistance Genes

This mechanism of gene transfer can occur for any gene, including ones that confer resistance to antibiotics. Another concern is whether or not the transplanted genes will become part of the recipient bacterium's DNA [3]. Because foreign DNA can introduce disruptive elements or jeopardize the genetic integrity of the bacterium, bacteria have defense mechanisms developed in to break down incoming genetic material. These systems, like as CRISPR-Cas and restriction-modification systems, aid in the defense of bacteria by recognizing and destroying foreign DNA before it has a chance to enter their genomes [38]. These defense mechanisms are not perfect, though. Certain foreign DNA can infiltrate bacterial cells and possibly inflict harm or transfer new features, such antibiotic resistance, by eluding detection or bypassing these defenses. This flaw in the defense systems of bacteria emphasizes the ongoing struggle between the bacterium and incoming genetic material during evolution.The likelihood of the incoming DNA being kept increases, if it is assimilated and benefits the bacteria. A bacterium that acquires an antibiotic resistance gene, for instance, would fare better than its susceptible neighbors and may proliferate if it is exposed to the antibiotic again [39].

3.8.2. Horizontal Gene Transfer

Resistant genes may be quickly and effectively transferred between populations through a process called horizontal gene transfer, which involves genetic alteration by microbes. In microbial populations, it is the most significant mechanism of resistance development and propagation. One of the main forces behind bacterial evolution is the acquisition of foreign DNA material by horizontal gene transfer (HGT), which is typically the cause of the emergence of antibiotic resistance. Conjugation, transduction, and transformation are the primary techniques [40].

3.8.3. Transformation

The term "transformation" describes a microorganism's capacity to use free DNA fragments from its environment. After being broken up into pieces, dead cell DNA leaves the cell [40].

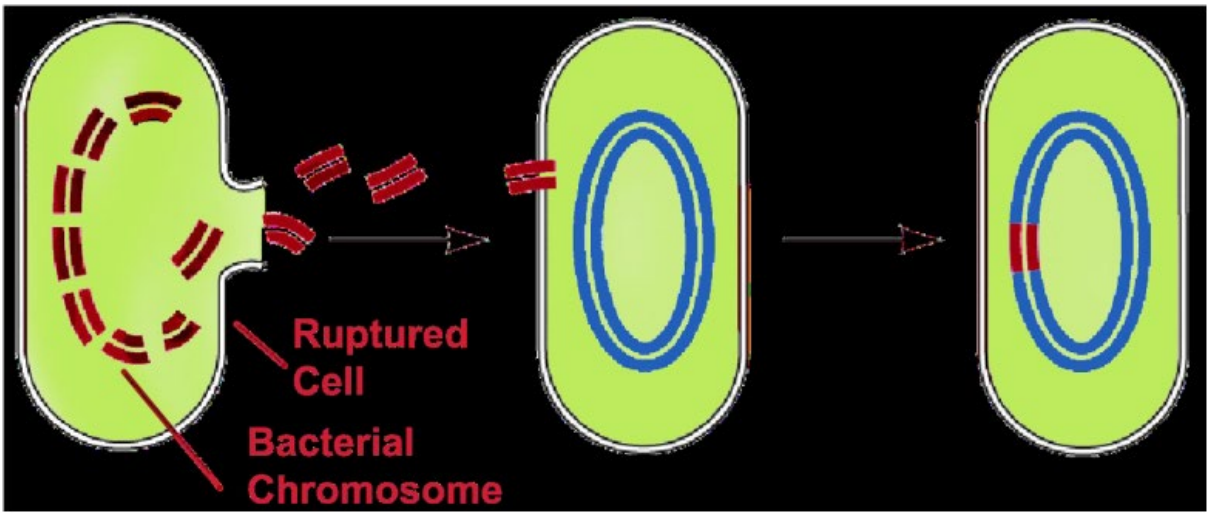

Figure 2: Transformation of Resistant Gene

Source: (Bbosa et al. 2014) [40].

3.8.4. Transduction

The process by which bacteriophages-viruses that feed on bacteria-transmit genetic information from one creature to another is called transduction. It's comparable to how diseases are spread from animal to animal via mosquitoes. But bacteriophages are more intricate than mosquitoes, which are passive carriers. Since viruses are themselves, they insert their genetic material into bacterial cells and greatly multiply there. Using the host bacterial cell's replication, transcription, and translation machinery, they typically reproduce by producing several verions, or whole viral particles, which include the viral DNA or RNA and the protein coat [40]. Low fidelity characterizes the packing of bacteriophage DNA, and the bacteriophage genome may be packed with tiny fragments of bacterial DNA. Simultaneously, several phage genes remain within the bacterial chromosome. Many additional bacteriophages are released into the environment by the finally ruptured cell, infecting other microbes [41].

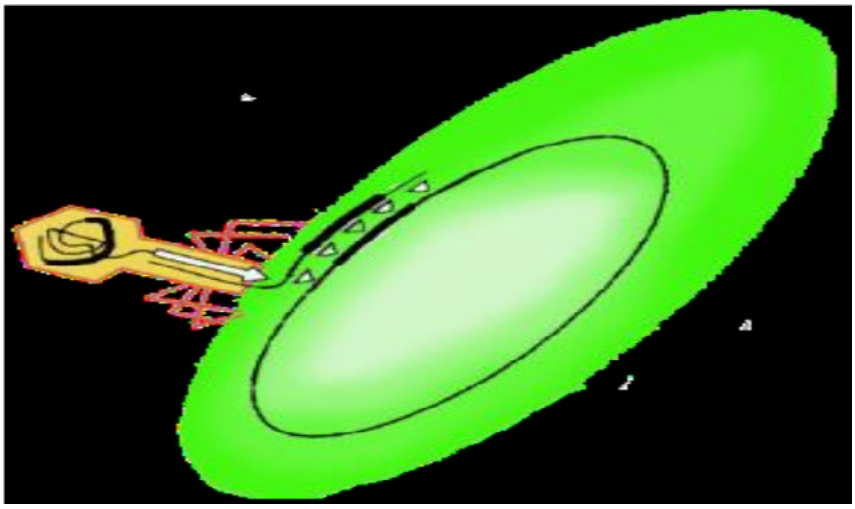

Figure 3: Transduction Mechanism (Bacteriophage)
Source: (Bennett 2008) [41].

3.8.5. Conjugation

The transfer of genetic material between bacterial cells by direct contact or a bridge-like link between two cells is known as bacterial conjugation. It is a horizontal gene transfer process, just as transformation and transduction are, except it doesn't require contact between cells. Since bacterial conjugation includes the exchange of genetic material, it is frequently thought of as the bacterial counterpart of sexual reproduction or mating. The donor cell supplies a conjugative or mobilizable genetic mobile element-typically a plasmid or transposon-during the conjugation process. This mechanism is extremely significant since it may happen effortlessly between various kinds of bacteria [42].

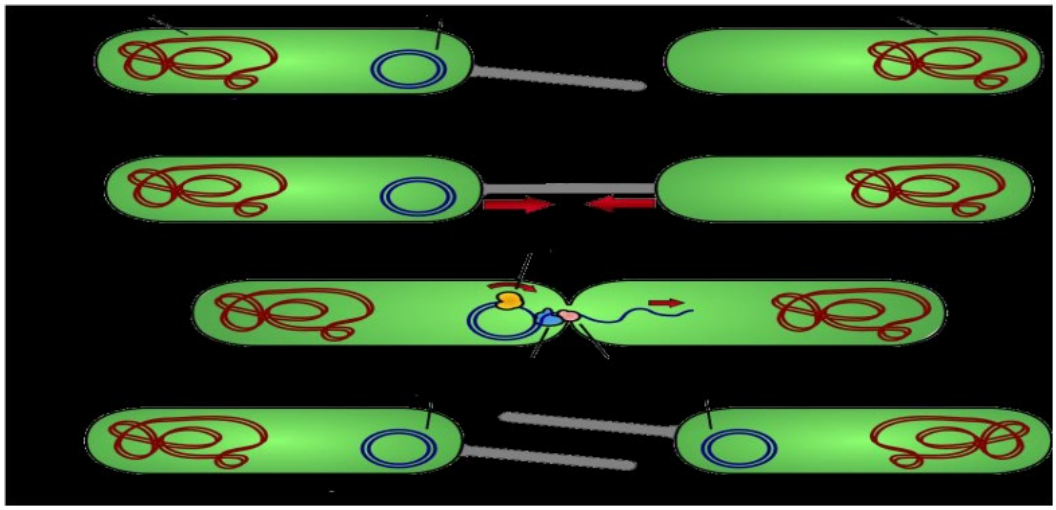

Figure 4: Conjugation Methods
Source: (Bockstael and Aerschot, 2009) [42].

3.9. Biofilm Formation

The micro colonies that make up biofilms are encased in a highly hydrated polymeric matrix and have interstitial spaces surrounding them that allow nutrients to move between the cells. Interstitial spaces between micro colonies can be thought of as a primitive circulatory system. Within biofilms, microorganisms grow into ordered communities with structural and functional variability like to that of a multicellular creature. Gene expression patterns of biofilm bacteria are altered by cell-to-cell communication molecules, also known as quorum sensing molecules. Such signals accumulate to levels high enough to activate genes involved in the differentiation of biofilms at a given population density [43].

Microorganisms form biofilms for survival, shielding them from host immune reactions and antimicrobial agents. Polymorphonuclear neutrophils adhere to, pierce, and produce cytokines in developing and fully developed cells under similar shear circumstances, as seen in Staphylococcus aureus biofilm [43]. Nevertheless, they are unable to clear the bacteria [44]. Actually, polymorph nuclear neutrophils' "frustrated" and failed attempts at phagocytosis may cause the production of cytotoxic and proteolytic chemicals that aggravate tissue and eventually cause prosthetic osteolysis [44].

Beyond the conventional routes of antimicrobial resistance outlined above, it is now acknowledged that development inside biofilms can further obstruct antimicrobial action. Compared to non-adherent planktonic cells, biofilms-well-assembled, surface-attached microbial communities-have a notably stronger resistance to antimicrobial agents due to their extracellular matrix (ECM) encasement [45,46].

Bacterial aggregation can reduce antibiotic susceptibility, independent of surface growth. Factors contributing to increased antibiotic resistance include restriction of antibiotic penetration by the extracellular matrix (ECM), secretion of antibiotic-modifying enzymes, accumulation of filamentous bacteriophages, differential metabolic activity, persister cells, biofilm-associated upregulation of bacterial efflux, enhanced horizontal gene transfer and mutation frequency, and interactions between different bacterial species within mixed-species biofilms. A classic example is the emergence of a plasmid carrying a vanA vancomycin resistance gene in a VRSA strain [47].

4. Spread of Antimicrobial Resistance and Public Health Significance

Antimicrobial resistance from livestock feedlots to the environment is distributed by a number of vehicles. The classic physical carriers are soil, water, crops, and animal protein (such as meat, milk, and fish). ARGs have recently moved from animal farms to urban environments thanks to livestock insects [48].The primary molecular means of ARG transmission in the environment is probably bacteriophages [49]. ARG spread has also been aided by airborne particulate matter from beef cattle feedlots [50]. These developing vehicles may pose greater health risks to people than conventional automobiles because control is a challenge.

Consuming tainted water, crops, shellfish, and animal protein exposes humans to antimicrobial resistance through the food chain. Direct contact during leisure and agricultural pursuits including swimming, plow work, and sowing can also result in exposure [51,52]. Workers at animal feedlots and farmers are more likely than the general population to develop antibiotic resistance. The development of resistant infections in the clinic may result through horizontal ARG transfer from ambient bacteria to human commensals and pathogens [53]. To limit the spread of ARG, the rate of routine acquisition of antibiotic resistance through food and the environment must be decreased. Numerous antimicrobials used in animal feed are also prescribed to treat illnesses in humans. High levels of antibiotic use in feed have disturbed public health officials and consumers due to the possibility of bacterial resistance in these animals' gastrointestinal tracts (GITs). Such resistance can also spread to bacteria in the gastrointestinal tract through the food chain [54].

The reservoir of resistant bacteria has significantly increased as a result of feeding calves, pigs, and poultry low doses of antibiotics like tetracycline and penicillin to encourage growth. These virulent bacteria may spread to humans from animals. With Salmonella infections, this is well known. Antibiotic-resistant bacteria can transfer from animals to people indirectly through food (such as when carcasses are tainted during slaughter), polluted water, animal waste, or, less frequently, direct contact (such as between farmers and slaughterhouse employees) [55].

5. Diagnostics to Novel Approaches

The conventional methods for evaluating antibiotic resistance in microorganisms have been bacterial culture and antimicrobial susceptibility testing. It is non-invasive, labor-intensive, inconclusive, and may overlook the majority of germs that are not cultivable. Currently used molecular approaches to identify antibiotic resistance genes include polymerase chain reaction (PCR), quantitative real time PCR (RT-PCR), multiplex PCR, whole genome sequencing, DNA microarray, and metagenomics and the characterization of AMR may increasingly make use of a cutting-edge method known as matrix-aided laser desorption ionization-time of flight mass spectrometry (MALDI-TOF-MS) [56].

5.1. Microscopy

Bacteria cultured on agar plates can be counted using microscopy techniques even before they have proliferated to the point where colonies are visible. For example the visible portion of E. Coli colonies has around 5,106 individual bacterial cell, however microscopy may already detect micro clusters of 120 cells [57]. Real-time growth curves and quantifiable bacterial counts may be seen using automated microscopy equipment. Fluorescence in situ hybridization (FISH)-based multiplexing automated digital microscopy (MADM) for fast online AST has been marketed by Rapid Diagnostics [58,59]. The Accelerate Phenol system may extract toxins from clinical samples (blood or urine) using a rapid electrophoresis process that embeds pollutants in a gel. After that, a change in the electric field's polarity forces the germs back into the liquid. Samples taken from the Mueller-Hinton media-growing bacterial culture exhibit a fluorescence signal every ten minutes [60]. Currently approved by the FDA, this system seems to be the only growth-based quick diagnostic AST technology available [61].

5.2. Micro Array

DNA microarrays use the well-known nucleic acid hybridization idea to work, making it possible to simultaneously identify and analyze thousands of genes' expression patterns in one test. When hundreds of different DNA sequences-from the virus or the host are physically attached to a silicon wafer or glass slide, a solid little checkerboard is produced [62]. Spotted DNA and high-density oligonucleotide microarrays are commonly used forms of DNA microarrays. They were initially created on glass slides with specific reference genes. Over the past two decades, DNA microarray technology has advanced significantly. A quick DNA-labelling technique based on biotinylated primers has been reported for disposable microarrays. Melting curve testing using a quick cartridge has been proposed for identifying Mycobacterium sp. resistant to pyrazinamide [63]. DNA microarrays have been used as genomic tools to identify drug-resistant genes in addition to being often used for gene monitoring and expression analyses. Using Identibac microarrays, AMR genes were discovered in gram-negative bacteria that were isolated from human feces, both anaerobic and aerobic [56].

5.3. Mutagenic Analysis

Metagenomics is a molecular approach that analyzes the DNA of microbial communities isolated from ambient materials without prior culture it has contributed to the illumination of a substantial correlation between AMR and microbiome by identifying complex microbial communities and their functional components implicated in AMR in bacteria in clinical and environmental samples [64,65]. In metagenomics, both function-driven and sequence-driven analytic techniques are applied. They are both based on next-generation sequencing techniques developed by commercial companies. For the sequence-driven approach, a large number of sequence reads are produced using sequence analysis tools [66]. Functional metagenomics has enabled the discovery of novel mechanisms of antibiotic resistance, mobilomes, and new antimicrobial resistant genes [67]. It is not necessary to have prior knowledge of these genes in order to discover novel ARGs in natural environments [68,69].

5.4. Molecular Techniques

The PCR method is a widely used genome amplification approach for identifying AMR strains. The clinical efficacy of genetic testing and evaluation has increased with recent developments in multiplex PCR and RT-PCR. The availability of ARG (antimicrobial resistance genes) targets is greatly impacted by changes in WGS(whole genome sequence). High throughput quantitative polymerase chain reaction (HT-qPCR) is a quick, simple, and efficient method for investigating antibiotic resistance (ARGs) in clinical and subclinical samples. It has been used to evaluate ARGs from various samples, such as Neisseria gonorrhoeae, which showed resistance to medications like cefixime, ciprofloxacin, spectinomycin, and azithromycin. However, due to its limited sensitivity, it cannot be used for diagnostic testing on clinical specimens [70].

5.5. Biosensor System

Biosensors are a cutting-edge invention in current advise that measure chemical or biological reactions by providing signals proportionate to the concentration of an analysis in the reaction. Antibiotic exposure causes observable changes in the morphology, metabolism, motility, mass, heat output, and concentration of nucleic acids in bacteria as well as their membranes. Heat generation and the pace at which new cells develop are connected, according to micro calorimetry techniques [71]. With the use of isothermal micro calorimetry, the microbial viability in biofilms was continuously monitored, regardless of the presence or lack of antimicrobials [72].

Biosensors are devices that are becoming more and more crucial

for early diagnosis and individualized treatment planning. Through effective device integration, fabrication, interface, packaging, and performance, nanotechnology has enhanced the sensing field. A transducer (amperometric, semiconductor, potentiometric, thermometric, piezoelectric, photometric) and a biocatalyst (bioreceptor) make up a biosensor. They can lessen pathogen multidrug resistance and enhance antibiotic stewardship; they are appropriate for point-of-care devices. According to recent research, real-time sensing devices based on nano-carbons may accurately and swiftly identify bacterial cells. These derivatives could be used in next-generation sensing devices. The most popular technique for assessing microbial cell reporters to find AMR strains is using biosensors [73].

6. Current Status of Antimicrobials Resistance and Public Health Importance in Ethiopia

A major worldwide health issue that impacts Ethiopian public health is antimicrobial resistance (AMR). According to Abebe and Birhanu, (2023) studies emphasized the alarming increase in antimicrobial resistance (AMR) in Ethiopia, which has been caused by a number of issues including improper use of antibiotics, inadequate infection prevention and control procedures, and restricted access to high-quality medical treatment [74]. The report stressed the critical need for concerted efforts by communities, legislators, and healthcare professionals to address antimicrobial resistance (AMR) through strong public awareness campaigns, antimicrobial stewardship initiatives, and surveillance. To counteract this escalating public health emergency in Ethiopia, research & development expenditures for novel antibiotics and substitute therapeutic approaches are also essential.

In Ethiopia antimicrobial resistance (AMR) has become a serious public health concern, especially when it comes to infectious diseases. Research has demonstrated that common bacterial pathogens, like Escherichia coli, Klebsiella pneumoniae, and Pseudomonas aeruginosa, Salmonella spp. and Staphylococcus aureus, which are commonly isolated from clinical, animal, and environmental sources, exhibit broad resistance [75]. The situation is made worse by the fact that multidrug-resistant (MDR) bacteria are highly prevalent; resistance rates for Staphylococcus species, Salmonella species, and Escherichia coli are 96%, 81%, and 77%, respectively [75]. This increase in AMR is mostly due to the widespread overuse of antibiotics in animal medicine, agriculture, and human healthcare. This presents a significant obstacle to the treatment of infectious diseases, raising national healthcare expenses as well as morbidity and mortality rates [76].

The development of resistant infections affects both the efficacy of medical care and the safety of the food supply, which has significant implications for Ethiopia's public health. Specifically, AMR poses a danger to the management of foodborne infections, which are common as a result of inadequate sanitation and hygiene standards [76]. It has been suggested that the One Health strategy be used to combat the AMR dilemma since it unifies the fields of environmental, animal, and human health [75]. Nevertheless, attempts to prevent the spread of AMR are hampered by the absence of well-coordinated surveillance systems and thorough data on its prevalence. The increasing prevalence of antimicrobial resistance (AMR) in Ethiopia poses a threat to public health and infection control efforts if left unchecked.

Antimicrobial resistance (AMR) in Ethiopia is best addressed with a One Health strategy, which acknowledges the interdependence of environmental, animal, and human health. The goal of this comprehensive framework is to coordinate efforts among several sectors in order to stop the spread of infections that are resistant. Since antibiotics are frequently used in animal production in Ethiopia, AMR is especially worrying in the agricultural sector. This practice contributes to the generation and spread of resistant bacteria that can infect humans directly or through the food chain [76]. The One Health strategy encourages cooperation amongst medical experts, veterinary professionals, environmental scientists, and legislators in order to put into practice efficient AMR containment techniques, such as better infection management, more responsible antibiotic usage, and increased public knowledge.

Ethiopia announced an updated AMR containment and prevention strategy plan in 2021 that is in line with the One Health paradigm. The goal of this revised approach is to improve surveillance systems for tracking patterns of antibiotic resistance and usage in the fields of environmental, animal, and human health. Improving laboratory capabilities, encouraging infection prevention and control (IPC) procedures, and supporting the prudent use of antibiotics in both clinical and agricultural contexts are among the top concerns [77]. The strategy plan also places a strong emphasis on creating rules that limit the over-the-counter selling of antibiotics, encouraging community involvement in AMR containment, and educating the public about the risks associated with antibiotic abuse. Ethiopia hopes to prevent the spread of resistant diseases throughout its interconnected ecosystems, thereby lowering the burden of antimicrobial resistance (AMR) and protecting public health [78].

7. Conclusion and Recommendations

Antibiotics are widely used in both human and animal health practices, treating diseases and feeding additives. However, their misuse, abuse, and overuse create conditions for the growth of antibiotic-resistant bacteria. Inefficient medications, non-laboratory focused antibiotic therapy, sub-therapeutic dosing, and inadequate drug storage contribute to these infections. Despite guidelines for responsible antibiotic use, misuse by healthcare professionals, untrained practitioners, and drug users continue. This rapid spread of resistant bacteria can increase animal mortality, illness, medical expenses, and productivity. Despite the significant impact of antibiotic resistance, there is insufficient surveillance and focus on prudent medication use to reduce it. The failure to create new antibiotics to fight multidrug-resistant bacteria is a concerning effect of this flawed system. Innovation in pharmaceuticals needs to move beyond incremental improvements to existing drugs and focus on creating new classes of antimicrobials, alternative therapies, and integrated solutions. Alongside this, public education on rational drug use is crucial in curbing the misuse of antibiotics, which is a major driver of resistance. Raising awareness among the

community, healthcare providers, and policymakers ensures that antibiotics are used judiciously, preserving their efficacy for future generations. Without emphasizing these twin strategies-innovative drug development and rational use-the fight against AMR cannot be fully effective. Awareness creation should be conducted on rational use of drugs for the community and other stake holders. Antimicrobial resistance (AMR) poses a significant threat to global public health, fueled by the overuse of antibiotics. To combat this, there is a critical need for innovative antibiotics and strategic approaches to drug development, as well as awareness campaigns promoting responsible antibiotic use [79,80].

Data Availability

All datasets that have led to the drawn deductions in the manuscript are here in presented in the paper and upon reasonable request, the corresponding author can grant access to the datasets used and/or examined in this study.

Conflict of Interest

The Author declared no conflict of interest.

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Ethical Consideration

The synthesis and analysis of previously published studies are part of this review on the causes, dissemination, and emerging diagnostic tools of antimicrobial resistance (AMR) in Ethiopia. As a result, neither the direct connection with human subjects nor the gathering of fresh data from humans is involved. Every study that was cited followed ethical standards and got the required clearances from ethics committees or institutional review boards (IRBs).

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