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Mechanisms of development of antimicrobial resistance in bacteria: A review

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Abstract
Treatment of infections with antimicrobial agents in clinical practice has been the most successful advances in the health care system. But now a day's treating bacterial infections become difficult due to their ability to develop resistance. The objective of this paper is to review the mechanisms of antimicrobial resistance development in bacteria. Bacteria may be intrinsically resistant to antimicrobial agents or may acquire resistance genes by de novo mutation or via a process of transduction, conjugation, and transformation. Mechanism of resistance may be through drug target alteration, drug modification, and reduced drug accumulation as a result of decreased permeability or active drug efflux. Resistant bacterial infections lead to increased morbidity, mortality, healthcare costs, and negative consequences for livelihood and food security. It is aggravated in developing than developed countries. Therefore, further study on mechanisms of resistant development to generate new insights of counteracting mechanisms is essential. In addition, awareness creation is important to prevent antimicrobial resistance development. More importantly, prudent use of available antimicrobial agents and the development of alternative new drugs are recommended.

Keywords: Antimicrobial, bacteria, misuse, mutation, resistance mechanism

Introduction
Microorganisms are essential components of the biosphere. They play a great role in the maintenance and sustainability of the ecosystem. But to survive, they have evolved mechanisms that enable them to respond through selective pressure exerted by various environments and competitive challenges [1]. They develop a mechanism against antimicrobials and it enhanced as a consequence of adaptation of infectious agents to antimicrobials exposure through continued over reliance on and imprudent use of antibacterial agents [1, 3]. Applications of antimicrobial agents in clinical, veterinary, husbandry, and agricultural practices are considered the major factor responsible for the emergence and spread of antibiotic-resistant bacteria [3].

A variety of microorganisms were identified to cause infectious diseases in the latter half of the 19th century. Antimicrobial chemotherapy has made a significant progress and during the 20th century resulting in the overly optimistic view that infectious diseases would be prevented and controlled in the near future. However, infections with drug-resistant organisms remain an important problem [4]. Marked increase in antimicrobial resistance among common bacterial pathogens is now threatening the successful use of any therapeutic agents [5-6]. Several important pathogens, including methicillin-resistant *Staphylococcus aureus* (MRSA), Vancomycin-resistant Enterococcus (VRE), multidrug-resistant (MDR) *Pseudomonas aeruginosa* and third-generation Cephalosporin-resistant *Escherichia coli* and *Klebsiella pneumonia*, poses a serious threat to public health [7].

Different bacteria react differently to antibiotics, either due to inherent differences such as unique cell envelope composition, or development of resistance [8]. Cross-resistance of different antibiotics may also occur due to common resistance mechanisms [2]. An intrinsic mechanism is solely due to the inherent microbial property of natural chromosomal genes and efflux system, but acquired mechanism involves genetic mutations or gene exchange methods [9]. Resistance to antimicrobials can be due to modification of a drug target, active efflux (or decreased entry), molecular bypass, and chemical modification of the compound [10].

Microorganisms respond by developing resistance mechanisms to antimicrobial exposure and currently antimicrobial resistance among bacteria, viruses, parasites, and other disease-causing organisms is a serious threat to infectious disease management globally [1].

Bacterial resistance results in treatment failure, which can have serious consequences, especially in critically ill patients; and also, with respect to the cost-containment pressures [11, 13]. Antibiotic resistance is estimated to cause around 300 million premature deaths by 2050, with a loss of up to $100 trillion to the global economy [6]. Several microbes have found ways to circumvent different structural classes of drugs and are no longer susceptible to most therapeutic regimens [14]. Fully understand the mechanisms of bacteria resistant to antimicrobials is very important to design novel strategies to counter the resistance threat. We need to develop antimicrobials with understanding that the microorganism will respond to them, and resistance will develop. Thus, efforts to develop antimicrobials and study mechanisms of resistance should be continuous, resilient, and steady [6].

Lack of basic knowledge on antimicrobials resistance mechanisms is one of the primary reasons that there has been so little significant achievement in the effective prevention and control of resistance development [5]. Understanding of mechanisms of drug resistance at molecular level is basic on new drug development and clinical use [10]. It is also important to define better ways to keep existing agents useful for a little longer and to help insight into innovative therapeutic approaches. Therefore, the objective of this paper is to review the mechanisms of antimicrobial resistance development in bacteria.

Antimicrobial agents and their mechanism of action

Antimicrobial drugs are agent that kill microorganisms or inhibit their growth [15]. They have been very important cornerstone of clinical medicine since the second half of the 20th century and have saved a great number of people from life-threatening bacterial infections [4, 16, 18]. Antimicrobials are mostly used veterinary drugs [19]. The unique advantages of the usage of antimicrobials in food animals are for the prevention and control of diseases, improvement of production performance, protection of the environment, and public health [12, 20]. In addition to their essential role in the clinic, antimicrobials are used in non-medical applications like promoting growth in livestock [18]. Most antimicrobials currently in use are produced by environmental microorganisms or are derivatives of these natural antibiotics [21].

Mechanism of action (MOA) of antimicrobials is based on their ability to interfere with the metabolic machinery of microbes. It is essential to understand how bacterial resistance develops [22]. Antimicrobials can be classified by their MOA as either inhibiting or interfering with cell wall synthesis, protein synthesis, nucleic acid synthesis, metabolic pathway, or the bacterial membrane structure [23].

The cell wall is a common requirement for most bacteria to cause infection. Some antimicrobials like penicillins and cephalosporins inhibit synthesis of the bacterial cell wall by interfering with the bacterial enzymes, peptidases required for the synthesis of the peptidoglycan layer [11, 22]. Nucleic acid synthesis inhibition occurs through antimicrobials that disrupt DNA and RNA synthesis by interfering with nucleic acid (e.g., quinolones, rifamycins) biosynthetic processes in the cell. In metabolic pathway inhibition, antimicrobials (e.g., sulfonamides) block bacterial biosynthesis of folic acid synthesis. Sulfonamides are structural analogues of p-amino benzoic acid (PABA). PABA is a substrate in the synthesis of tetra hydro folic acid, a donor of one carbon units in the synthesis of purine and pyrimidine nucleotides. Sulfonamides competitively compete for the dihydropteroate synthetase enzyme active site and block the formation of nucleotide precursors [24]. Rifampicin interferes with a DNA-directed RNA polymerase. Quinolones disrupt DNA synthesis by interference with topoisomerases and DNA gyrase during replication [11].

Inhibition of protein synthesis occurs by aminoglycosides and tetracyclines which bind to the 30S subunit of the ribosome thereby weakening the ribosome-tRNA interaction, whereas chloramphenicol binds to the 50S subunit blocking peptidyl transferase reaction. Disruption of bacterial membrane structure (e.g., polymyxins) exert their inhibitory effects by increasing bacterial membrane permeability, causing leakage of bacterial contents and death of the bacterium [11]. Figure 1 shows the different classes of antimicrobials and their respective mechanism of action [25].

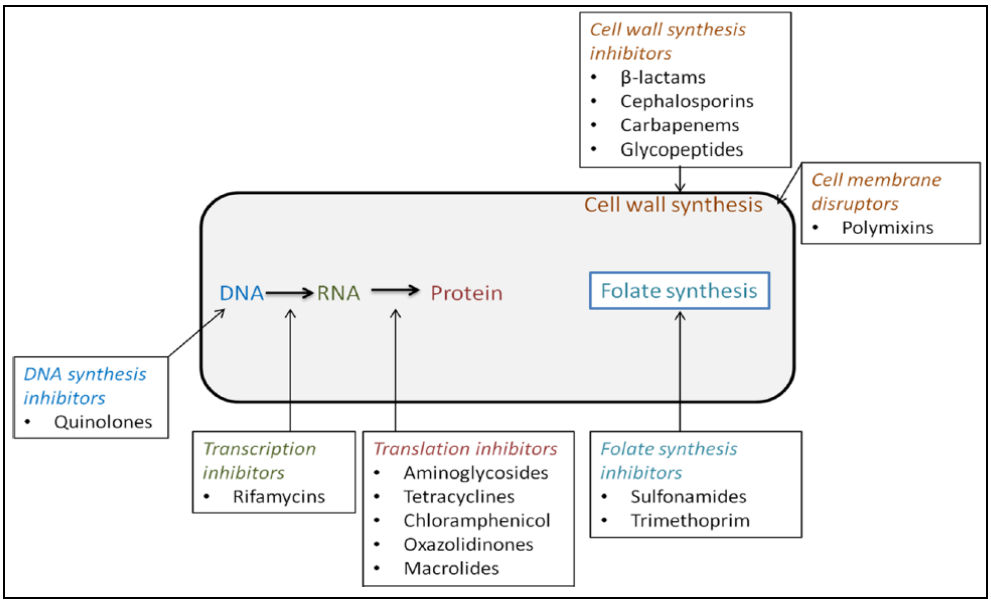

Fig 1: Different classes of Antimicrobials and their mechanism of action

Antimicrobial resistance

Resistance is an organism's ability to remain viable and multiply under a concentration of antibacterial agents that would inhibit other members of the strain [8]. Thus, it limits the treatment options and increases cost and potential side effects for patients [27]. The emergence of drug resistance has always been a major concern worldwide right after the introduction of drugs for common use [9]. Environmental microorganisms which produce antibiotics have systems to avoid the activity of the antibiotics they produce, and these elements can be resistance genes upon their transfer to pathogenic microorganisms [21]. The first drug resistance occurred was against penicillin by *S. aureus* and the resistance began to be noted within two years of its introduction in the mid1940s [9, 28]. Failure to successfully treat infections due to antimicrobial resistance leads to more severe or prolonged illness, death, production losses and negative consequences for livelihoods and food security [12].

Causes of antimicrobial resistance

Infection is one of the leading causes of death in developing countries. This is mainly related to the emergence of new source of infection, especially due to antimicrobial resistance [29]. The main risk factors which cause antimicrobial resistance are microbial behavior and antimicrobial usage [27].

Microbial behavior: Microbial organisms are often present in large populations in an infection site. As an individual organisms, they contain limited genomes that cannot survive all conditions. However, as a population, they contain a wide range of genes that can respond to various environments. Compounded with their rapid generation time, they can rapidly select for the sub-populations that can best survive when taking antimicrobials [27]. The microbial world includes organisms whose direct ancestors were present at the beginning of life. The success of microbial life and become more life on this planet can be linked directly to the microbial ability to rapidly and effectively adapt to environmental change [30]. Adaption is a very essential condition for the survival of microorganisms [9]. Genetic variability of microbial organisms occurs by mutation and genes that may be mobilized within and between microbial species through the varied mechanisms of horizontal gene transfer [31]. Among the mobile genes that have been characterized, many have shown to provide solutions to many different adaptive challenges such as resistance to antimicrobials [9, 32]. Bacteria become resistant to ensure their survival against antimicrobial agents [1].

Antimicrobial usage: Antimicrobials have been instrumental in reducing mortality and morbidity associated with bacterial infections [23]. However, any imprudent practice in drug production, distribution, prescription, dispensing, and finally consumption of the drug by the patient may result in the emergence of resistance [33]. Antimicrobial resistance has been increasing at an alarming rate due to the widespread use of antimicrobials and inappropriate utilization of them has provided a strong selective environment that favors the appearance and maintenance of resistant bacteria [9, 19]. Misuse of antimicrobials has been associated with increased rates of antimicrobial resistance (AMR) and increased healthcare expenditures. It is the single most important cause of resistance [9, 34]. The uncontrolled application of antimicrobials in agricultural industry and animals is also becoming the main factor for resistance development [9]. The intensive use of antimicrobials in animals may promote the fixation of antimicrobial resistance genes in bacteria, which may be zoonotic or capable to transfer these genes to human-adapted pathogens or to human gut microbiota via direct contact, food, or the environment [17].

Antimicrobial agents inhibit susceptible organisms and select resistant ones. The genetic resistance determinant in resistant bacteria is selected by the antimicrobial drug [35]. Drug resistance emerges when these components come together in an environment or host, which can lead to a clinical problem [36]. Thus, the driving forces of emerging antimicrobial resistance are repeated exposure of the bacteria to antibiotics and access of the bacteria to a large resistance gene pool as it may be available in a polymicrobial environment [37, 38]. Improper prescribing of antibiotics in patients with viral infections and overuse of broad-spectrum antibiotics have resulted in the emergence of resistance [23]. Antibiotic resistance is the result of natural selection for resistance-conferring mutations, and it is important to understand the evolutionary processes underlying this selection. The evolution of resistance, resistance-conferring mutations have a cost on bacterial fitness (growth rate). Most mutations come at a price. However, bacteria can acquire additional mutations known as compensatory mutations that offset those costs and help maintain resistance mutations in a population [18].

Antimicrobial resistance is aggravated in developing than developed countries due to gross abuse in the use of antimicrobials [33]. Poverty is linked to increasing resistance rates, due to several selection pressures. The first is the limited supply of medications, resulting in healthcare providers being forced to prescribe partially or non-efficacious treatments. And poorer countries are more likely to allow antimicrobial dispensing without a prescription, and often have less precise manufacturing standards [27]. With the abuse of antimicrobials, antimicrobial resistance has increased dramatically. This is critical, especially in developing countries where they are not only misused but are often underused due to financial constraints [39].

Africa bears the greatest infectious disease burden from AMR [34]. Antimicrobials sold in Africa are of questionable in pharmacological quality. Adverse climatic conditions such as high ambient temperatures and humidity affect the overall quality of the antimicrobials during storage [33]. Although Ethiopia has not yet conducted a large-scale drug resistance study, it has been reported that among pathogens such as *Escherichia coli*, *Shigella spp*, *Salmonella spp*. and *Staphylococcus aureus* are in a trend of increasing resistance rates to commonly prescribed antimicrobials [39]. In Ethiopia, the misuse of antimicrobials, unskilled practitioners, inappropriate prescription practices, inadequate patient education, limited diagnostic facilities, unauthorized sale of antimicrobials, lack of appropriate functioning drug regulatory mechanisms, and inadequate surveillance coupled with the rapid spread of resistant bacteria contributed to AMR [33, 34, 40].

Types of Antimicrobial Resistance

Bacterial resistance can be described in two ways: bacteria may be intrinsically resistant to antimicrobial agents or may acquire resistance by de novo mutation or via the acquisition of resistance genes from other organisms [11].

Intrinsic resistance: It is an innate characteristic of bacteria that renders it naturally resistant to an antimicrobial agent due to naturally occurring genes which found on the bacterial

genome that could generate a resistance phenotype [5, 14, 23]. It is genus or species-specific, and microorganisms naturally do not possess target sites for the drugs. Therefore, the drug have no effect on them, or their permeability to the active substances is naturally low. It may be due to the differences in the chemical nature of the drug or the microbial membrane structures mainly for those that require entry into the microbial cell to affect their action [1, 37]. In such cases, all strains of that bacterial species are resistant to all the members of those antibacterial classes [11]. Intrinsic resistance can also be referred to as insensitivity due to the invulnerable nature of the organism toward that drug [9]. Resistance to penicillin G expressed by most Gram-negative bacteria is due to the complexity of its cell wall has an outer membrane that Gram-positive bacteria do not have. In addition, all Gram-negative bacteria have inherent resistant to vancomycin due to their cell wall structure being different from that of Gram-positive cell walls. However, this intrinsic form of resistance is not a major source of concern for human and animal health [12].

Acquired resistance: It represents a strain-specific property and a naturally susceptible bacteria acquires ways of not being affected by the drug. It can acquire resistance genes from mobile genetic elements that carry one or more resistance genes and from the antibiotic producer bacteria. The resistance genes that antibiotic producers harbor as a mechanism of self-defense from their products are usually located in the chromosomal DNA [1, 37]. Acquired resistance may be based on mutations in certain chromosomal housekeeping genes which act as targets for antimicrobial agents [14]. Such mutations are mainly based on the exchange of one or a few bases and it causes a slight change in the amino acid sequence of the corresponding gene product. These sequence alterations often have little or no influence on the biological activity of gene products but render them insensitive to the inhibitory activities of the respective antimicrobial agents [37]. In de novo mutation, development of resistance is a matter of time because mutations occur randomly at a low frequency, and it can sometimes result in advantageous characteristics that can be selected. A given large number of bacteria in an infection cycle and in rapid generation time, the intrinsic rate of mutation is about 1 in 10^7 than a pool of 10^{10} bacteria would have mutations on average in a thousand loci. If one of those mutations confer resistance to an applied antibiotic, all sensitive bacteria are killed and the resistant one will grow, fill the space vacated by its dead neighbors and become the dominant variant in the population [12, 28].

The exchange of resistance genes among bacteria between members of a mixed bacterial population has distinctly accelerated the widespread occurrence of certain resistance genes in a large number of pathogenic bacteria. Resistance genes were usually first present in the bacteria in which they had evolved and were initially only transmitted vertically [37]. However, the mobility of resistance determinants among bacteria has been well known [11] and, when they integrate into mobile genetic elements, the resistance genes spread by horizontal transfer among bacteria of the same and of different species and genera. It is the principal mechanism for the rapid spread of antibiotic-resistance genes in bacterial populations. Genes that reside on plasmids may also further segregate within transposons that can actively cut themselves out of one DNA locale and insert into other locales, promiscuously moving their antibiotic resistance gene [28, 37].

Horizontal gene transfer results in the rapid spread of antimicrobial resistance genes between bacteria of the same and different species with the help of mobile genetic elements carrying one or more resistance genes [37]. Determinants of resistance are transmitted through plasmids, bacteriophages, transposons, and other mobile genetic material. Plasmids are extra chromosomal elements and capable of replication independently of the host chromosome [14]. They have resistance properties, and they can give the bacteria some survival advantages under certain conditions, such as the ability to survive in the presence of an antimicrobial agent [41]. Plasmids can carry one or more antimicrobial resistance gene(s) and they may integrate into the chromosomal DNA or can act as vectors for transposons and integrons/gene cassettes which enable them to move on their own from one host cell to another. Such plasmids are referred to as conjugative plasmids [37, 42].

Transposons have no replication system, so they must be integrated into replication-proficient molecules such as chromosomal DNA or plasmids in the cell to maintain stability [37]. These pieces of DNA contain terminal regions that participate in recombination and specific protein(s) (e.g., transposals or recombinase) to help access specific regions of the genome. It is responsible for the movement of the element which have no target specificity and therefore can insert themselves at various positions in the chromosomal or plasmid DNA and they are responsible for antibiotic resistance [14, 37]. Integrons contain collections of genes (gene cassettes) [14]. Gene cassettes differ from plasmids by the lack of replication systems, and from transposons by the lack of transposition systems. They move by site-specific recombination [37].

Bacteria resistance to antibiotics effects increase in bacterial population's size and can transfer their resistance genes to new generations (progeny) or to other bacteria [23]. Microbial resistance has its basis at the genetic level which is modified either by gene knockout or insertion [9]. Genes responsible for antimicrobial resistance can be transferred horizontally through horizontal gene transfer (HGT) mechanisms between bacteria of the same or different species and genera [6, 37]. The exchange can be accomplished through transduction, conjugation, and transformation [14].

Transduction essentially requires a virus-specific vector carrying a drug resistance gene for further introduction into bacterial host. Whereas conjugation is facilitated by plasmids through forming a temporary pilus between two adjacent bacteria for genetic material exchange [9]. It is transfer of DNA that occurs between live bacterial cells and requires direct contact between the donor and the recipient cell [43]. Mobile genetic elements (MGEs) can also serve as a vehicles to share valuable genetic information. Transformation is probably the simplest type of HGT. It is the process of taking foreign DNA from the environment and using it develop resistance [6]. These processes of antibiotic-resistant (Ab^r) gene exchange mechanisms are described at Figure 2 below [14].

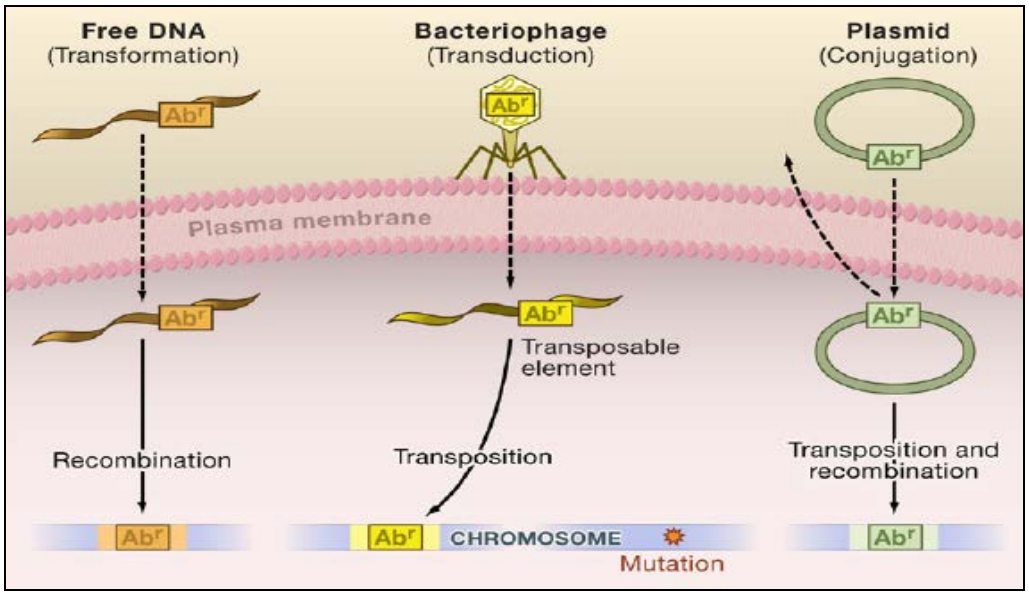

Fig 2: Acquisition of Antibiotic resistance

Mechanisms of resistance

Decreased permeability: Reduced intracellular accumulation of antimicrobials can be achieved in principle in two different ways: decreased uptake or increased removal of the drugs [37]. For antibiotics to exert their bacteriostatic or bactericidal actions on bacteria they must access intracellular targets [44].

Drug uptake may be decreased in gram-negative bacteria through the permeability barrier of outer membrane [37]. It is exclusion of antimicrobials from the cell, and it is an intrinsic resistance of gram-negative bacteria [2]. To circumvent this permeability barrier, these organisms have developed porins (such as OmpF in *E. coli* and OprD in *P. aeruginosa*) that function as nonspecific entry and exit points for antibiotics and other small molecules [14]. Those proteins are outer membrane proteins (OMPs) involved in the transport of antibiotics and the export of extracellular virulence factors [45]. Some bacteria can manipulate their cell wall or membrane to protect themselves from foreign drugs. For example, some Gram-negative bacteria can decrease the uptake of some antibiotics like aminoglycosides by altering the membrane porin frequency, size, and selectivity [9]. Alterations of porins through mutations cause reduction or shift in the type of porin expression, structural alteration, impairment, or even loss of porins by which antibiotics enter the bacterial cell [37].

A more general example of impermeability leading to resistance can be found in the biofilm mode of growth that seems to predominate in bacteria in nature [46]. Several bacterial species like *P. aeruginosa, K. pneumoniae, S. aureus,* and *M. tuberculosis* have been shown to form biofilms [25]. Biofilm is defined as a thin layer of microbial communities that interlock with each other on organic or inorganic surfaces and enclosed by their secreted matrices of extracellular polymeric substance (EPS) [26]. Biofilm formation is a multistage process in which microbial cells adhere to the surface and the subsequent production of an extracellular matrix which results in a firmer attachment and anchoring them to one another [47]. The binding of cells with the protective matrix increases the time required to suspend cells in the antimicrobial agent and the formation of microbial biofilms is an important reason for failure of antimicrobial therapy [8, 46]. Cells in a biofilm (sessile cells) are phenotypically and physiologically different from free-swimming, non-adhered (planktonic) cells. One of the typical properties of cells in a mature biofilm is that attached bacteria exhibit enhanced resistance to antimicrobial agents due to their ability to withstand antibiotic concentrations that would normally kill planktonic cells. Consequently, bacterial biofilms significantly contribute to morbidity and mortality in patients [47, 48].

Several mechanisms are thought to be involved in biofilm resistance, including slow penetration which can be affected by ionic interactions between the glycocalyx and the antimicrobial agent, an increase in the distance the agent must diffuse, molecular sieving (size exclusion), and the viscosity of the glycocalyx [8, 26]. Rates of horizontal gene transfer through mobile genetic elements are typically higher in biofilms than in planktonic cultures [47]. Bacterial cells located in different parts of the biofilm community may respond differently to antimicrobial agents. In the microenvironment of biofilms, there is a possible anaerobic condition because of oxygen concentration is limited in the center of biofilms compared with at the surface [26].

Bacterial growth in biofilms will slow or stop due to a limited nutrient environment, which is generally accompanied by an increase in resistance to antimicrobial agents [26]. The barrier to drug penetration formed by the exopolysaccharide, limited diffusion and the low growth rate of bacteria in biofilms were related to drug resistance [8]. The synergy between a low-permeability outer membrane and active efflux from the cell is very important for antimicrobial resistance of bacteria [2].

Efflux pumps: The production of complex bacterial machineries that can squeeze toxic compounds out of cells can also result in antimicrobial resistance [6]. Efflux or pump out the drugs is the active removal of antimicrobials from within the cell and it is a highly efficient mechanism of resistance. Antimicrobials to be effective they must reach their specific bacterial targets and accumulate at concentrations that can act in some reasonable time frame [10, 28]. Bacteria become resistant to tetracyclines if they overproduce membrane proteins that act as an export or efflux pump for the drug. The drug is pumped out faster than it can diffuse in, so intra bacterial concentrations are kept low and ineffectual; bacterial protein synthesis proceeds at largely unimpeded rates [28]. These pumps expel drugs from the cytoplasm limiting their ability to access their targets [18]. In

addition to antimicrobials, these pumps export biocides, dyes, detergents, metabolic inhibitors, organic solvents and molecules involved in bacterial cell-cell communication [2].

Most drug efflux proteins belong to five distinct protein families [9]. These include the small multidrug resistance family (SMR) [49]; the resistance-nodulation-division (RND) family, which is part of the larger RND permease superfamily; the major facilitator superfamily (MFS); the ATP-binding cassette (ABC) family; and the multidrug and toxic compound extrusion family (MATE). All of these transporters catalyze active drug efflux and therefore require energy, mostly in the form of proton motif force and in the form of ATP [2].

The efflux mechanism of RND systems is with a cytoplasmic membrane pump which can contact the continuous channel, and with the membrane fusion protein presumably assisting in the interaction of these other two components. This makes the extrusion of antimicrobial agents to the surroundings more direct and efficient, bypassing the periplasmic space [50]. The presence of genes encoding RND pumps in bacteria varies widely, ranging from none in *Mycobacterium tuberculosis*, one in *Bacillus subtilis*, four in *Escherichia coli* to ten in *P. aeruginosa* [45].

Multidrug and toxic compound extrusion (MATE) and small multidrug resistance (SMR) are structurally similar to MFS but are designated as distinct families, based on phylogenetic diversity (MATE) or size (SMR) [2]. Except for ABC transporters, efflux by proteins of the above-mentioned families is driven by proton motive force and is known as secondary transport. On the other hand, the primary ABC transporters drive efflux through ATP hydrolysis [9, 14]. Figure 3 illustrates the main types of bacterial drug efflux pumps in well-characterized representatives of these families from Gram-positive and Gram-negative bacteria in *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Lactobacillus lactis* respectively [2].

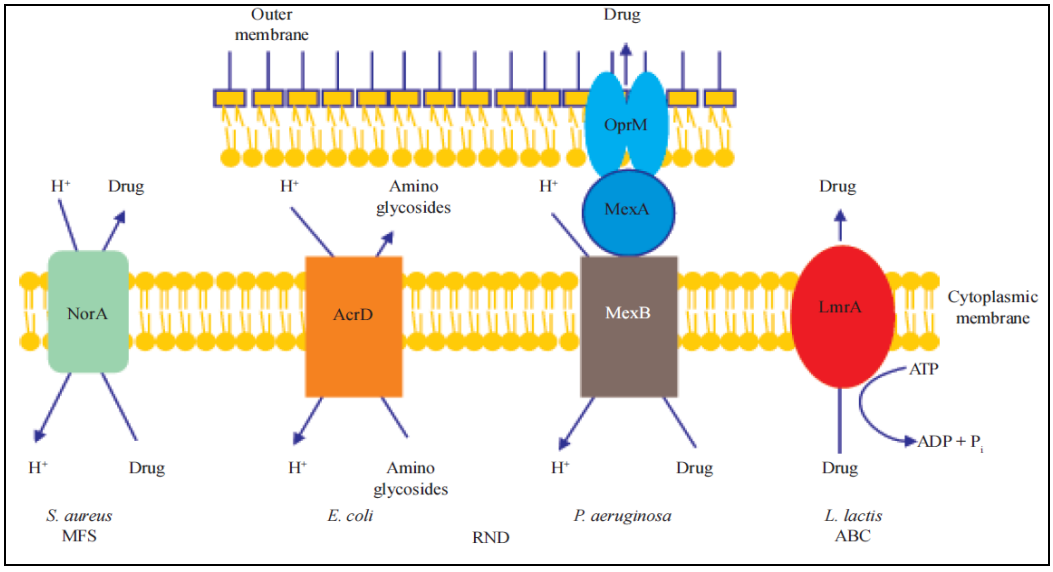

Fig 3: Schematic illustration of the main types of bacterial drug efflux pumps

NorA is a member of the major facilitator superfamily (MFS); AcrD and MexAB-OprM, two members of the resistance-nodulation-division (RND) family and LmrA, a member of the ATP-binding cassette (ABC) family [2].

RND family transporters are most commonly found in Gram-negative bacteria [51]. Functional OprM is a trimeric protein, the typical barrel configuration of the outer membrane-embedded portion of the protein exhibits long periplasmic extensions capable of spanning the periplasm. The outer membrane protein component, OprM and the RND transporter component, MexA and MexB involved, and it is conceivable that OprM directly interacts with MexB [49]. Since gram-negative bacteria have two membranes separated by periplasmic space, it is now generally considered that RND efflux system in these bacteria functions as a three component system, enabling the bacterium to squeeze out antibacterial from the cytoplasm to the extracellular medium without a need of outer membrane channels. Therefore, inhibition of efflux pumps is an attractive way to improve the efficacies of antimicrobials as a substrate of these pumps [52].

Inactivation of antimicrobial agents: One of the most successful bacterial strategies to cope with the presence of antibiotics is to produce enzymes that inactivate the drug by adding specific chemical moieties to the compound or that destroy the molecule itself, rendering the antibiotic unable to interact with its target [6]. Destroying or manipulating the active component of the antimicrobial agents has always been considered as one of the effective techniques adopted by microbes for protection [9]. Enzymes that modify antibacterial drugs are divided into two categories: for example, β-lactamases that degrade antibiotics and others (including the macrolide and aminoglycoside-modifying proteins) that perform chemical transformations [14].

Destruction of the antibiotic molecule is an enzyme-catalyzed inactivation and the evolution of such highly efficient catalysts is evidence of the strong and sustained selective pressure of antibiotic action. The first evidence for such enzymes was described in the 1940s with the discovery of the penicillin inactivating β-lactamase activity [6, 10]. These enzymes destroy the amide bond of the β-lactam ring, rendering the antimicrobial ineffective [6]. Bacteria that produce lactamase secrete this enzymatic weapon into the periplasm to destroy β-lactam antibiotics, penicillins and cephalosporins before they reach the PBP targets in the cytoplasmic membrane. A single β-lactamase molecule can hydrolyse 10^3 penicillin molecules per second, which is an effective strategy [28, 44].

Chemical alterations of the antimicrobials through the production of enzymes capable of introducing chemical

changes to the antimicrobial molecule is a well-known mechanism of acquired antimicrobial resistance in bacteria [53]. The most frequent biochemical reactions they catalyze include acetylation, phosphorylation and adenylation. One of the best examples of drug modification resistance is the presence of aminoglycoside modifying enzymes (AMEs) that can covalently modify the hydroxyl or amino groups of aminoglycoside molecule. AMEs with their biochemical activity, they are called acetyltransferase, acetyltransferase, or phosphotransferase [6].

Modification of target site: A common strategy for bacteria to develop antimicrobial resistance is to avoid the action of the antimicrobial by interfering with or modifications of the target site [6, 12]. Microbial uptake of an antimicrobial drug is essential for a target-oriented action [9]. An interaction between an antibiotic and a target molecule is very specific so even small changes in a target molecule can influence antibiotic binding to a target and it may not fit or may render the target site inaccessible to the antibiotics [37, 54]. Modification of the molecular target is the prominent mechanism of bacterial resistance. This can arise through point mutations in the genes encoding the target site, enzymatic alterations of the binding site, complete replacement or bypass of the original target site resulting in relatively rapid resistance where such changes have a minimal impact on microbe fitness and it can arise through highly efficient and selective modifications [6, 10]. Well-characterized examples of mutational resistance involve the development of resistance in rifampin (RIF) and fluoroquinolones (FQ) [55, 56]. RIF is a rifamycin that blocks bacterial transcription by inhibiting the DNA-dependent RNA polymerase. RIF binding pocket is a highly conserved structure located in the β subunit of the RNA polymerase (encoded by rpoB), and after binding, the antibiotic molecule interrupts transcription by directly blocking the path of the nascent RNA [55]. RIF resistance has been shown to occur by point mutations resulting in amino acid substitutions in the Rpob gene [57]. FQs kill bacteria by altering DNA replication through the inhibition of DNA gyrase and topoisomerase IV. Development of chromosomal mutations in the genes gyrA-gyrB and parC-parE encoding subunits of the DNA gyrase and topoisomerase IV respectively, are the most frequent mechanism of acquired resistance to these compounds [56].

In target bypass microbes have mechanisms that circumvent antibiotic action by evolving 'work around' that is not antibiotic sensitive [10]. Bacteria produce an alternative target that is resistant to inhibition of antimicrobials. For example, Methicillin resistance in *Staphylococcus aureus* (MRSA) produces an alternative PBP due to the acquisition of chromosomal cassette *mec* element. The *mecA* gene responsible for methicillin resistance encodes the β-lactam-insensitive protein PBP2a which enables cell wall biosynthesis to occur despite the native PBP (penicillin-binding protein) being inhibited in the presence of antibiotics [6, 58].

Current status of antimicrobial resistance in Ethiopia

Antimicrobial resistance has become a major health concern both in developing and developed countries [39]. The rates of resistant microorganisms which complicate the management of healthcare-associated infections are increasing worldwide and getting more serious in developing countries [59]. The causes of AMR in developing countries are complex and may be rooted in practices of health care professionals and patients' behavior in the use of antimicrobials. The problem is more pronounced in developing countries due to limited antibiotic options, irrational drug use, poor drug quality, poor sanitation, malnutrition, poor and inadequate health care systems, and lack of control for antibiotic use and stewardship programs [33].

Microorganisms resistant to antimicrobial is a major challenge to the health system and high levels of AMR detected to widely used antibiotics in East Africa [60, 61]. Studies in East Africa have shown that high levels of AMR to commonly used antibiotics in Gram-negative bacteria, 50% – 100% resistance to ampicillin and trimethoprim or sulfamethoxazole [62]. A situation analysis of AMR in Ethiopia also indicated high resistance levels and it remains a huge concern in the progress of treating infectious diseases [60, 63]. AMR research is gaining increasing attention within Ethiopia, and it is a good trend to combat AMR infections [34].

In Ethiopia, the available reports indicate a trend towards increasing resistance rates among pathogens such as *Escherichia coli*, *Shigella spp*., *Salmonella spp*. and *Staphylococcus aureus* to commonly prescribed antibiotics, including ampicillin, amoxicillin, penicillin, tetracycline and trimethoprim/sulfamethoxazole [39]. Recent studies conducted at Jimma university medical center indicated that the most common identified isolates were *Escherichia coli* (24.6%), *Klebsiella species* (23.8%), and *Staphylococcus aureus* (20.6%). From these, 30.2%, 41.3%, and 19% were multidrug-resistant, extensively drug-resistant, pan-drug resistant respectively [59]. In another study, *S. aureus* isolates were detected among primary school children and prisoners in Jimma town in Ethiopia, and the overall prevalence of MRSA was 23.08%. The prevalence of MRSA among primary school children and prisoners was 18.8% and 48% respectively. The isolated *S. aureus* and MRSA displayed multiple drug resistance (MDR) to 2 to 10 antibiotics [64].

Report of antimicrobial resistance profiles of bacterial isolates from meat and meat products in Ethiopia showed that the bacterial isolates have different antimicrobial resistance profiles against selected drugs. Approximately 25% were *Salmonella spp*. Which was resistant to ampicillin. In addition, 9% of *Salmonella spp.* and 2% of *E. coli* O157:H7 were resistant to ceftriaxone. Comprehensive estimate showed that 10% of *E. coli* O157:H7 isolates were resistant to ciprofloxacin. Moreover, *Salmonella spp*. (6%), *L. monocytogenes* (5%) and *E. coli* O157:H7 (2%) were resistant to gentamicin. It is relatively high as compared to other countries and hence, there is a need to design intervention to ensure meat safety in the sector [65].

Ethiopia is committed to joining global partners in the detection and prevention of AMR. To prevent and contain AMR, Ethiopia has established a national monitoring network [60]. A coordinated effort is needed from all stakeholders working in health system to tackle antimicrobial resistance in Ethiopia [59]. The Ethiopian Drug Administration and Control Authority in 2009 conducted a baseline study, antimicrobial use, resistance, and control to understand the resistance status of Ethiopia. The study examined changes in resistance to various pathogens, including *Streptococcus pneumoniae*, *Salmonella spp*. and *Staphylococcus aureus* between 1996 and 2000 in Ethiopia. *S. pneumoniae* showed an increase in resistance to erythromycin from 0% in 1996 to 19.2% in 2000 [60]. In other studies, reported from different parts of Ethiopia also indicated that increasing rates of resistance in *E. coli*, *Shigella spp*., *Salmonella spp*. and *S. aureus* to commonly prescribed antibiotics such as ampicillin, amoxicillin,

penicillin, tetracycline and trimethoprim or sulfamethoxazole [39, 66].

Following the 2009 situation analysis, Ethiopia focuses on disseminating information about AMR to the community and establishing a national strategy and action plan to combat AMR. The National Advisory Committee on Antimicrobial Resistance Prevention and Containment was established in 2011 from both the human and animal health sectors of the government and then, the National Strategic Framework for prevention and containment of antimicrobial resistance had been developed and endorsed. And also, in 2015 five-year strategy for the prevention and containment of antimicrobial resistance for Ethiopia was developed [60]. AMR surveillance was launched based on the findings of the 2009 situation analysis, involving multi-stakeholder working groups in July 2017 at four sentinel sites across the country. This surveillance initiative was one of the first systematic efforts to prospectively collect, analyze, and report national-level microbiology results from a network of hospitals and public health laboratories in Ethiopia [60, 67].

Techniques for combating resistance

Responsible and prudent use of antimicrobials

The golden era of antibiotics led to the discovery of several important antibiotics, but the last decade has yielded very few potential drugs on account of various reasons. This has set the alarm bells ringing as we may be soon out of antimicrobials to treat drug-resistant infections [25]. Presence of potential resistance mechanisms to new or old antibiotics unless new understanding and technologies discovered the pre-antibiotic era awaits our descendants [5]. The widespread misuse and improper drug dispensing and handling practices can affect the drug quality and it can contribute to the development of drug resistance [19]. The occurrence of a high number of resistant strains are due to the use of sub-therapeutic level of antibiotics and/or short treatment in the dairy farms [68]. Monitoring rational use of drugs and periodic assessment of the antimicrobial sensitivity of drugs prior to use are essential [69]. Since we have a limited set of antibiotics, we have to use techniques to prevent the occurrence of resistance. The minimal and rational use of the available antimicrobial drugs should be encouraged in veterinary practice. There are better ways of dosing and combining drugs to increase efficacy and decrease resistance [18, 70].

A pair of synergistic antibiotics are more effective than the sum of the efficacies of each antibiotic when used alone [18]. Treatment with combinations of inhibitory compounds that have different modes of action is also important [5]. In individual treatment, correct dosage is important to minimize the development of resistance. Even though optimal dosing regimen requires costly *in vivo* experiments, modeling, and understanding the kinetics of how the drug and target interaction occurs, and then the best time and concentration parameters for an antibiotic dose can be predicted [71]. Antibiotics should be developed as a class separate from broad-spectrum agents as niche-specific [5].

Research on the quality of currently used antimicrobials is also urgently needed since many antimicrobials dispensed in developing countries are of questionable in pharmacological quality. So, to prevent or at least delay antimicrobial resistance strict regulatory controls on antimicrobials delivery, accurate prescription practices through improved diagnosis, and controlled therapeutic use in human, animal husbandry and agriculture are required [5, 33, 72]. One strategy to reduce the development and spread of antimicrobial resistance is to reduce selection pressure by limiting or eliminating the use of antimicrobial drugs. This is based on the assumption that resistant microorganisms will be outnumbered by susceptible strains if the selective advantage of possessing the resistance determinant is diminished [72].

Raise awareness on antimicrobial resistance

There is a general lack of awareness among food animal owners about the correct use of antibiotics [19]. Enhancing education to make awareness about how antimicrobial agents work and under which conditions antimicrobial resistance develops and spread are very important to implement measures that counteract resistance development and to design control strategies [73, 74]. Lack of knowledge in antimicrobials may significantly affect the quality of prescribing. Physicians with inadequate knowledge may be more willing to prescribe the widest possible range of treatments. A better understanding of the mechanisms of resistance will help clinicians regarding for proper usage of antimicrobial agents [16]. All stakeholders should raise awareness in minimizing the occurrence of resistance development and thereby transmissions of resistant pathogens [75]. The creation of public awareness about the occurrence of antimicrobial-resistant is very important for their prevention [69]. Increase national awareness on AMR through public communication programs or promote and support the establishment of multi sectoral coalitions to address AMR at local or national level [12].

Promote new and rapid diagnostic methods

A multi-factorial approach must be taken to counteract the rising rates of AMR and continued investigation is necessary to acquire new knowledge about the basic mechanisms of resistance, gene transfer and adaptive bacterial evolution. This includes studying the effect of persistence and host-pathogen interactions and their contribution to antimicrobial resistance which may be helpful for development of new drugs and diagnostic tests [27, 76].

Today, antimicrobials are rarely prescribed based on a definitive diagnosis. So, having rapid, low-cost, and readily available diagnostics is very important to mitigate this problem. Rapid diagnostics will reduce unnecessary use of antimicrobials [12]. The resistance of microorganisms to traditional antibacterial drugs is increasing and pharmaceutical companies are reluctant to invest in research and development of novel antimicrobial agents necessitate the exploration of alternative therapies [77].

Promote the development and use of alternatives

Ideal alternatives to antimicrobials should be non-toxic with easy elimination from the body, stable through gastrointestinal transit, easily decomposed and environment friendly, selectively active against pathogens with minimum or no effect over resident gut flora, improve feed efficiency, and be free from resistance [78]. Areas of alternative therapeutic strategies, include small molecules to attenuate bacterial virulence and disrupt biofilm formation, bacteriophage therapy, identification of drug targets that select for reduced bacterial fitness during development of antimicrobial resistance, and enhancement of host immune responses through vaccination and immunomodulation [72, 79]. The use of bacteriophage is emerging as an alternative treatment option for bacterial infections. Bacteriophage therapy is a necessary alternative to conventional antibiotics

[80]. They are bacterial viruses with the capacity to invade bacterial cells and induce lysis of the bacteria [33].

The application of vaccines as a valid alternative therapeutic to antimicrobials has attracted much attention since resistance is not observed against vaccines, because a vaccine enhances the body's natural defenses, while an antibiotic operates separately from the body's normal defenses. Nevertheless, new strains may evolve that escape immunity induced by vaccines [78, 81]. Veterinary vaccines are an ideal candidates for animal health and welfare and food production as they help in preventing infection; reducing consumption of antimicrobial drugs; enhancing food productivity and mitigating the impacts of drug resistance. Vaccination can reduce the rise of antimicrobial-resistant strains by decreasing their use [82, 83].

Pumping antimicrobial out of cells is a common feature of most environmental and pathogenic microbes and it is the most widespread form of resistance. Devising compounds that interfere with efflux of active inhibitors from the cell is an attractive strategy for the design of modified therapeutics [84, 85]. Revival of old antimicrobial agents, including those discarded, should be re-investigated. Combinations of antimicrobial agents with an inhibitor (e.g., an efflux inhibitor) should be explored for their ability to restore the activity of old antimicrobial agents [73].

Conclusion

Drug discovery was a relief. It has been instrumental in reducing mortality and morbidity associated with bacterial infections. However, bacteria become smarter toward massive usage of antimicrobials and result in developing resistance to antibacterial drugs. The development of resistance is a normal adaptive response of bacteria. It is the major cause of health concern since drug resistance rate exceeds the development of new drug alternatives. It increases at an alarming rate due to many contributing factors like misuse of antimicrobials, low drug quality, limited diagnostic facilities, and lack of functioning drug regulatory mechanisms coupled with rapid spread of resistant bacteria. Bacteria can develop resistance to antimicrobials by mutating existing genes, or by acquiring new genes from other strains or species. They become resistant through modulating membrane permeability, efflux pump mechanism, target alteration, and drug modification. Understanding the development of antimicrobial resistance is crucial to develop means of preventing resistance to antimicrobial therapy. There are many reasons for antimicrobial resistance. So, single, isolated interventions have limited impact and coordinated action is required to minimize the emergence and spread of antimicrobial resistance. Treatment failure will cause suffering for the affected individuals and will cause treatment costs in animal and human health care. It has also a negative impact on community livelihoods and on national and global economies. Therefore, appropriate drug regulatory mechanisms should be functional, awareness creation to all stakeholders on the mechanisms of resistance development, prudent use of available antimicrobial agents such as clinician should diagnose properly and prescribe an appropriate drug of which a much selective and narrow-range and further study on the mechanisms of resistant development, antimicrobial discovery and alternative therapies should be enhanced and supported for better understanding and designing of control strategies.

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