CHAPTER

Antimicrobial resistance mechanism and the developing management strategies

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Abstract

The antimicrobial resistance mechanism gives rise to serious global threat for life of human as well as animals. It is not confined to the clinical pathogen but also widely prevalent in the environment. Different bacterial species have acquired resistance gene due to different mechanisms like modification of genome by mutations followed by the transfer of resistant genes by plasmids or through various types of self-resistance mechanisms. This chapter deals with recent advancement in this field to understand the mechanism of development of resistance in microbes and the treatment of antibiotic resistant pathogens by plant bioactive compounds, which help to prevent resistance and effective treatment against multi resistant microbes, and their combination effect with antibiotics on multi resistant microbes by different biotechnology aspects and use of different strategies to combat antibiotic resistance mechanisms in microbes
Keywords: antibiotics, antimicrobial resistance, infectious agents, resistance mechanisms, treatment, MDR, re-sensitizing, bioactive compounds

Introduction

Over 100 years there is drastic development occurs in the field of antibiotics and increases the life span of humans by 23 years. The antibiotic discovery started in 1928, by penicillin and from that it is beginning of golden age of natural product antibiotic discovery which take hike in mid 1950s **(Hutchings et al., 2019).** But unfortunately, the researchers were unable to continue and maintain this process of antibiotic discovery due to emerging of resistant pathogens leads to end of this era. The termination of production of new antibiotics because of non-judicious use of antibiotic are the important factors affiliated with increasing of antibiotic resistance strains of bacteria **(Aslam et al., 2018).** Antibiotics formed as with different generic drugs and are of low cost and easy access leads to misuse and overuse of antibiotics. Large numbers of antibiotic resistant bacteria are evolving due to reason of selective pressure **(Fernandes & Martens, 2017).**

The prediction of UK government commissioned O'Neill, that about 10 million people will die due to antibiotic resistant infection by 2050 **(O'Neill, 2014).**

Development of antibiotic

The development of drugs and the concept of chemotherapy was recognize by Paul Ehrlich, who invented synthetic arsenic based pro drug salvarsan for the treatment of *Treponema pallidum*, the causative agent of syphilis **(Gelpi et al., 2015).** The first broad spectrum antimicrobials are sulphonamides which were discovered for clinical use and are still used today; it is penicillin which was observed by Alexander Fleming on a contaminated Petri dish in 1928 **(Fleming, 1929).** Norman Heatley, Howard Florey, Ernst Chain and colleagues at Oxford purify penicillin and lead to the development of penicillin as a drug (**Abraham et al., 1941).** Louis Pasteur, before the discovery of penicillin proposed that microbes had the ability to secrete material that kills other bacteria. By 20^{th} century it was reported that bacteria produce different types of diffusible and heat stable compounds, which had utility in treating and combating different types of diseases (**Frost, 2018**).

Three antibiotics, which were approved in the late 1990s and in the early 2000s are telithromycin, temafloxacin and trovofloxacin have serious adverse events and failure of product

The antibiotics discovered between 1945 and 1978, 55% came from the genus Streptomyces (**Embley & Stackebrandt, 1994**). Several theories have been proposed to explain why soil microbes make so many bioactive NPs. The long term infection and spread of antibiotic resistance bacteria leads to decrease production of new antibiotics which creates threatening situations of ineffective treatment against bacterial diseases. Different natural strategies are developing and introducing for effective antibiotic alternatives, in which agricultural applications or natural products were encouraged **(Stanton, 2013**).

Past times when antibiotic resistance were considered to be as biological costly trait. Resistant bacteria were handicap without antibiotic selection, gene mutation or extra genes in competition with sensitive bacteria but now different research suggests that antibiotic resistance gene persist stably with or without antibiotic selection **(Andersson & Hughes, 2011**). Start of antibiotic development and research has created vast development in strategies to solve the global problem of antibiotic resistance **(*WHO | Global Action Plan on AMR*, n.d.).** WHO in partnership with the Drugs for Neglected Diseases initiative had launched the Global Antibiotic Research and Development Partnership for development of new antibiotic against antimicrobial resistance, and to increase the use of treatments for optimal conservation.

Selection of antibiotic-resistant bacteria and criteria for the prioritization of antibiotic-resistant bacteria (**Tacconelli et al., 2018) .** Members of WHO (World health organization) and ten international experts in clinical microbiology, public health, infectious diseases, and pharmaceutical research and development selected by WHO in august, 2016. Members of these

groups selected 20 bacterial species which had about 25 patterns of acquired resistance based on WHO surveillance report on antibiotic resistant bacteria (**Garner et al., 2015**)**.** Criteria for the selection are those bacteria which cause chronic infections and require extended treatment courses. There are 10 selected criteria like community burden, mortality, 10 year trends of resistance, treatability, health care burden, transmissibility, preventability in health care and in community, prevalence of resistance and pipeline. Fungi, protozoa, viruses, helminthes and parasites were not included in this list **(Marsh et al., 2016).**

It's being a serious public health concern for medical department as the return of pre-antibiotic era because of high antibiotic resistant cases was observed (**Davies & Davies, 2010a**)**,** either it is community acquired or hospital acquired infections due to MRSA (Methicillin Resistant S. aureus), VISA (Vancomycin Intermediate Staphylococcus aureus), VRE (Vancomycin Resistant Enterococci) or ESBL (extended spectrum b-lactamase) enzyme producing Gram negative bacteria **(Kumar et al., 2013**)**.** The effective antibiotics were no longer available which can effectively and completely treat this type of bacterial infection with other multiple resistant bacterial pathogens like Streptococcus pneumonia, Mycobacterium tuberculosis, Pseudomonas aeruginosa and S. aureus, etc. **(Gupta & Birdi, 2017).**

The origin of antibiotic resistance gene by natural processes, it may be through bacteria having resistance gene of antibiotics use this as a mechanism of protection for themselves or development of resistance due to spontaneous mutations in chromosome of bacteria. The frequency of spontaneous mutation is about 10^{-8} – 10^{-9} **(Tiwari et al., 2011).** Once the emergence of antibiotic resistance gene occurred in the bacteria, the mutated gene will directly transfer to its progeny during process of replication. As the wild type of bacteria are killed in selective environment of antibiotics while the resistant mutant was allowed to grow and transferred **(Gupta & Birdi, 2017).** The ESKAPE pathogens, as name suggests, are capable of 'escaping' the cidal action of antibiotics **(Pendleton et al., 2013).**

DIFFERENT MECHANISMS OF ANTIBIOTIC RESISTANCE ACQUIRED BY BACTERIA

1. GENETIC BASIS OF ANTIMICROBIAL RESISTANCE

Genetic plasticity is the property shown by bacteria which help them in their existence in environmental threat and also in the presence of antibiotic compounds. Bacteria are sharing the same ecological environment with antibiotic-producing organisms and have developed the mechanism to resist harmful antibiotic molecules which help them to grow in that environment of antibiotics.

Major genetic strategies used by bacteria to acquire a resistance mechanism include:

A. Mutation- The bacterial cell which is susceptible to antibiotics shows a mutation in its susceptible gene which results in the survival of the non-resistant bacterial population in presence of antibiotics. Further, the susceptible bacteria will be eliminated from the population

followed by increase the number of antibiotic-resistant bacteria. Such mutations alter the antibiotic action results in antimicrobial resistance by mechanisms like- (i) Antimicrobial targets modification (decreasing the drug affinity) (ii) Decrease in the uptake of drugs by microbes (iii) Efflux mechanism which removes the harmful molecules (iv) Modulation in regulatory networks by changing in the metabolic pathways. So, the resistance mechanism that arises by mutational changes is diverse **(Munita & Arias, 2016).**

Bacteria itself attain mutations that result in multidrug resistance and have had a clinical relevance **(Strachan & Davies, 2017**). As technology develops in today's world and improvement in molecular techniques, it is easier to detect mutation easily. Mutation in 23s rRNA may increase the resistance as it has the binding site for antibiotics and inhibition of transcription and translation. For example, Clarithromycin resistant in *H. pylori* prevents the treatment of pneumonia and skin infections because of mutation in its 23s rRNA gene **(Alonso et al., 2014).** The extensive use of antibiotics also leads to resistance in bacteria. For example, extensive use of linezoid in clinical treatment has led to resistance in *S. aureus* and *Streptococcus pneumonia* and mutation in 23s rRNA in *Staphylococcal* species is responsible for reduced susceptibility to linezoid **(Gu et al., 2013).**

B. Horizontal Gene Transfer- Possession of the foreign DNA through HGT is important for the evolution of bacteria and also responsible for the antimicrobial resistance. In clinical practice, the natural products are used for the formulation of antibiotics. As the bacteria share the same environment with these natural products and there is strong evidence which proposes that "environmental resistome" is a great source for the acquisition of antibiotic resistance gene in clinical bacteria by harboring intrinsic genetic determinants of resistance and shows resistance for particular antibiotics.

Bacteria acquire the genetic material through three strategies that are Conjugation (sexual reproduction in bacteria) Transformation (acquiring of naked DNA), Transduction (Phage mediated). Mobile genetic elements (MGEs) are used by bacteria for conjugation as a vehicle to transfer the genetic information, therefore directly transfer from chromosome to chromosome **(Manson et al., 2010).**

Plasmids and transposons are most important MGEs, both of these plays an important role in evolution and distribution of antimicrobial resistance gene in the clinical microorganism. It was also studied that Integrons and site-specific recombination are also powerful mechanisms which play crucial role in the inheritance of resistant gene **(Munita & Arias, 2016)**.

2. SELF RESISTANCE MECHANISM

For the treatment of infectious diseases, the discovery of antibiotics is the biggest success in the field of chemotherapy but wide use of antibiotics will increase the chances of antibiotic resistance in bacterial strains **(Davies & Davies, 2010b).**

Naturally, the microbes show a low degree of antibiotic resistance in the original host but the microbes become opportunistic pathogen in immune-compromised person, the resistance may also be attained by microbes through a genetic mechanism through mutation in antibiotic targets or through a genetic transfer of plasmid which has an antibiotic-resistant gene, example: plasmid-encoded specific efflux pumps (such as TetK and TetL of *S. aureus*) **(Peterson & Kaur, 2018)**. The understanding of the molecular mechanism and biochemical basis might help find the management of antibiotic resistance mechanism **(Blair et al., 2015; Munita & Arias, 2016)**

1. **Biofilm formation**

Biofilm is the aggregation of microorganisms in which cells that are encased within a matrix of extracellular polymeric substances adhere to cell surfaces. Biofilm formation is the critical property adapted by bacteria for their successful colonization and pathogenesis. Quorum sensing is a cell to cell communication that is responsible for the formation of Biofilm by the detection of extracellular auto inducers. Biofilm has the ability to show resistance against antibiotics because of increased cell density as compared with planktonic cells. As quorum sensing is responsible for more antibiotic resistance, so inhibition of the communication process between the cells is one method to prevent the spread of antibiotic resistance **(Ali et al., 2018).**

2. **Intrinsic resistance**

All the physical conditions like pH, environmental changes, radiations, or change in intensity of light, all contributes to resistance in bacteria **(Russell, 2003).** But consideration will be given to the intrinsic mechanism of resistance attained by bacteria through natural means. The intrinsic ability of resistance attained by bacteria is known as insensitivity. In this, the organisms show a high level of resistance in whether it is exposed to that drug or not **(D'Costa et al., 2011)**.

All species and strains have their individual and unique phenotype and genotype to antimicrobial-resistant organisms. Study shows that expression of *BcnA* gene responsible for increased antibiotic resistance against the hydrophobic antibiotic. The transcription of a gene increases in the condition of antibiotic stress; this is responsible for increase in the level of intrinsic resistance **(El-Halfawy et al., 2017).**

STRATEGIES TO COMBAT THE MENACE OF DRUG RESISTANCE

Use of natural compounds

Natural products like plant-derived analogs are also a source of drugs or drug template and acquire very low toxicity that can diminish the drug-related issues during a prolonged time **(Baldwin et al., 2015).** The widely used natural derivatives are Flavonoids (Isocytisoside Eucalyptin- Pigmented compounds found in fruits and flowers of plants which include flavone, flavanones, flavanols, and anthocyanidins). These compounds show activity against multidrug-resistant strains of *Pseudomonas aeruginosa, S. Typhi, E. coli, K. pneumoniae*. There is a decrease in the stability of the bacterial membrane by an increase in the permeability of the membrane **(Chandra et al., 2017).**

Secondary metabolite like alkaloids consists of heterocyclic nitrogenous compounds which show broad-spectrum antimicrobial activity against different microbes like *P. aeruginosa, E. coli, S. aureus, S. mutans, M. gypseum, M. canis and T. rubrum* **(García et al., 2012).**

Secondary metabolites

Different plants produce large range of compounds which may not be important for their primary metabolism, but help plants increasing their adaptability to adverse abiotic or biotic environment conditions. These organic compounds are known as secondary metabolites which are biologically active compounds; they may be either an intermediate or end products as they are resultant of secondary plant metabolites **.** Secondary metabolites are diverse biochemical group of substances produced by the plant cell through secondary metabolic pathways that are derived from the primary metabolic pathways **(Mccreath & Delgoda, 2017**)**.** These metabolites act as a defence mechanism by interrupting between the metabolic pathways like cell signalling or with molecular targets in microbes or herbivores and some may show protection against oxidative stress or UV stress **(Wink et al., 2012).** About 2000 secondary metabolites are isolated and identified till now **(Kessler & Kalske, 2018),** and they classified according to their biosynthetic pathway and chemical structure. There are three main groups of phenolic (biosynthesized from shikimate pathways, containing one or more hydroxylated aromatic ring), alkaloids (non-protein nitrogen-containing compounds, biosynthesized from amino acids, such as tyrosine) and terpenoids (polymeric isoprene derivatives and biosynthesized from acetate via the mevalonic acid pathway) **(De Filippis, 2016).** These all groups consider as 90 percent of secondary metabolites, other minor group includes carbohydrates, saponins, essential oils, ketones, lipids, and others **(Anand et al., 2019).**

Mechanism of secondary metabolites on microbes

Plant secondary metabolites mode of action is depending upon their properties and chemical structures. They also have the ability to affect the microbial cell and their function in many ways like it shows interaction with membrane protein (ATPases and others), it shows effects on cytoplasmic membrane structure and function by disrupting the membrane (including the efflux system), leakage of ions by destabilization of the protein motive force, they also show interruption on DNA and RNA synthesis and also affects their functions, interruption in quorum sensing that is cell to cell communication **(Khameneh et al., 2019)**.

Quinones are secondary metabolites that contain aromatic rings having a ketone group as a substituent, there are around 400 naturally occurring quinones which are found today by researchers in all the parts of the plant **(Sher, 2004)**. *Hypericum perforatum* is a plant which contain Hypericin, an anthraquinone is an effective compound and show good antimicrobial activity against MRSA (Methicillin-resistant Staphylococcus aureus) and MSSA (Methicillin-sensitive Staphylococcus aureus) **(Dadgar et al., 2006).**

Alkaloids derived from an amino acid and it comprised of organic heterocyclic nitrogen compounds. Alkaloids grouped into three major classes that are Proto alkaloids, True alkaloids and Pseudo alkaloids. Alkaloids shows great effect on cell death and cell division because of having the ability to intercalate with DNA **(Savoia, 2012).** Cortex phellodendri, Rhizoma coptidis and Berberine are alkaloids containing antimicrobial activity against Streptococcus agalactiae. Berberine is found in Berberis spp was reported to had a mechanism of anti-herpes, by inhibition of synthesis of herpes simplex viral DNA and also found to have an ability of disrupting the membrane of bacteria because of its intercalating property **(Peng et al., 2015) (Chin et al., 2010).**

Mechanism of action of plants constituents act on microbes

Bioactive compounds from plant used for therapeutic use and for medicinal purpose because of presence of secondary metabolites. Antimicrobial activity of plant compounds varies according to the position of substituent groups, its structure and number linking groups, and climatic condition of the places where they grows.**(Assob et al., 2011)).** Antimicrobial compounds from plant targets different microbes like bacteria, fungi, viruses protozoans, etc. some compounds reflects antimicrobial with antimicrobial resistance modifying activity and some also show synergistic behavior with existence antibiotics. It was notified that chemically complexed compounds works more effectively compared to synthetic drugs because of having very minimal chances of developing resistance and fewer side effects **(Ruddaraju et al., 2019)**.

Bioactive compounds show different targets in microbes like it affects cytoplasmic membrane of bacteria by modifying its structure, permeability and functionality. Quorum sensing is the communication method of microbes, compounds may also have the ability to inhibit the quorum sensing gene and decrease their metabolic processes. Biofilm is one of the mechanism of increasing cause of MDR in microbes which became very difficult for different antibiotics to treat the infection, plant active compounds also helpful in inhibiting the formation of biofilms. They are also helpful for inhibiting viral replication by interfering viral protein and their interactions ((**Vaou et al., 2021)**.

There are different methods to identify bioactive compounds present in the plant extract by through combining both chemical and biological method. Combination of Mass spectroscopy and gas chromatography that is GC-MS technique is the best method to achieve our goal. Nuclear magnetic resonance is another technique for identifying bioactive molecule in the mixture of compounds present in the plant extract. NMR is also helpful in identifying mechanism of different antimicrobial compound and also for characterizing plant secondary metabolites **(Gallo et al., 2014).** HPLC is also a method for the identification of bioactive compounds and to establish metabolomics profile **(Kohler et al., 2016).**

Different strategies use to combat microbes by use of plant product

1. **Advancement in the medicinal plant by combination with antibiotics**

Modified antibiotics with a combination of plant extracts has shown great effects on the MIC (Minimum inhibitory combination) of antibiotics, as it works synergistically and show slightly decrease in MIC. The synergy is a key factor for medicinal plant extract by enhancing the separate contribution of bioactive compounds and antibiotics. This combination effect of plant extract with antibiotics known to be as resistance-modifying activity (RMA) and it depends on different factors like pharmacodynamics and pharmacokinetics. Pharmacodynamics are synergistic, additive and antagonist effect while pharmacokinetics effect is increasing the bacterial cell permeability for antibiotics or by affecting transporters, metabolism, absorption of antibiotics metabolizing enzymes **(Aiyegoro et al., 2009).** Quantitative identification of the synergistic test which is the combination of multiple compounds which are acting on the same target/receptor in a fixed ratio is calculated by combination index (CI) **(Chou, 2010)**. Synergy occurs by different mechanisms like degradation of antimicrobial enzymes, by inhibition of different steps in the biological pathway, by increasing the uptake of antimicrobial compounds through cell wall or by interaction of antimicrobial to cell wall (**Pillai S.K et al., 2005)**. Antogonism occurs when both bacteriostatic and bactericidal antimicrobial occur, or antimicrobial act on same site or act with each other. Modulation of compounds or drugs can increase the synergy and enhances the absorption of drugs by many different ways like delay of barrier recovery, or disruption of transport barrier, or by through reduction of excretion by inhibiting drug effects.

Combinations of plant essential oils and antibiotics show great results against antimicrobial resistant strains of bacteria. *Chrysanthemum coronarium, Origanum compactum, Melissa officinalis,* Boiss, *Origanum majorana,* and *Thymus willdenowii* plant extract in combination with antibiotics *ticarcillin, gentamycin,imipenem*, and *tobramycin,* against ten Gram-negative and Gram-negative bacterial strains showed synergy in some cases, but also an antagonistic effect against different bacterial strains was found **(Moussaoui & Alaoui, 2015)**. Some combination is also shows effect against biofilm formation. Essential oil with antibiotic norfloxacin is most effective for the inhibition of biofilm. **(Rosato et al., 2007).**

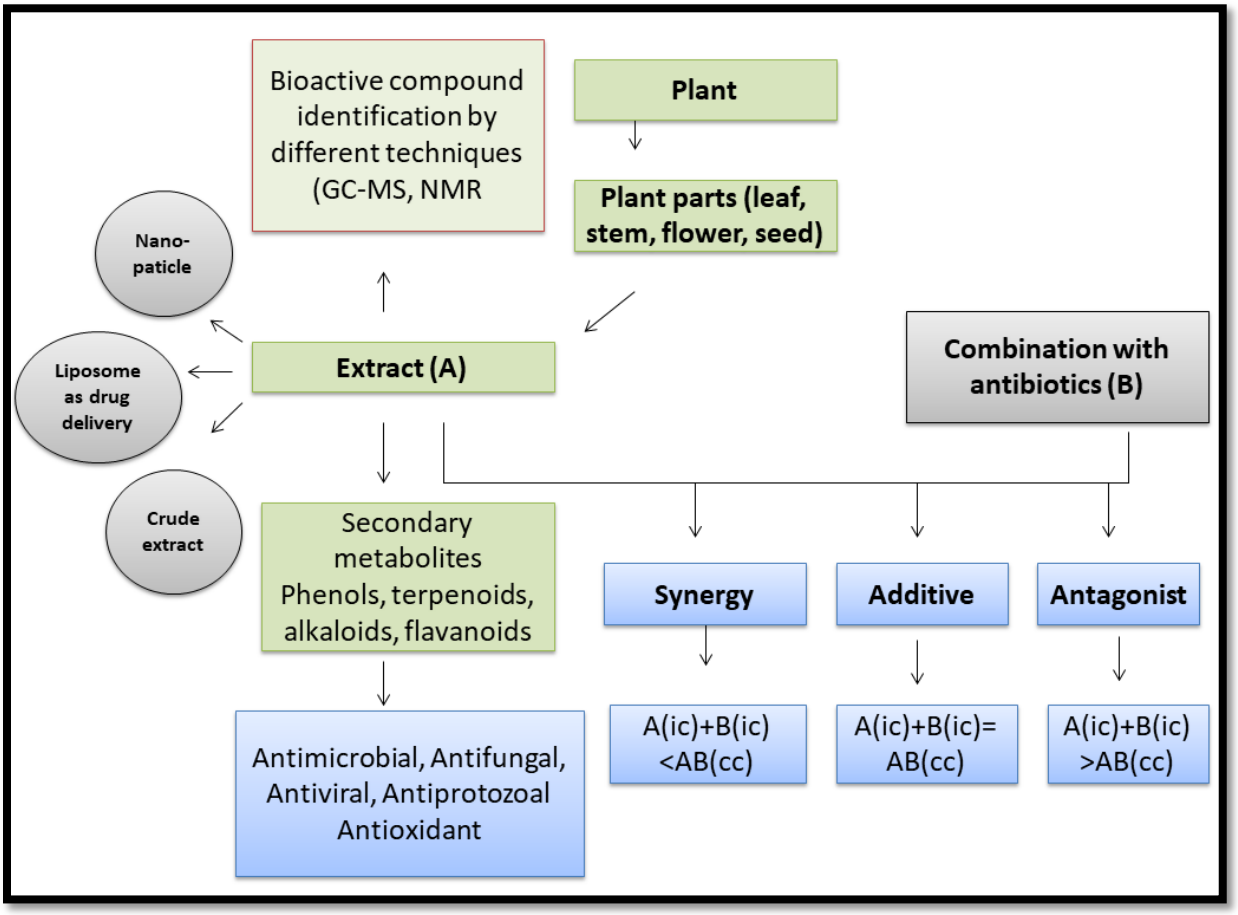

Fig: Different methods used by natural compounds to inhibit microbes

2. **Nanoparticle-based delivery of drugs, AMPs, and essential oils:**

The enormous use of nanomaterials in the field of biomedical applications magnifies the research interest and identifies the antibacterial mechanism of Nanoparticles **(Bayda et al., 2018).** Nanoparticles can alter the metabolic activity of bacteria as this particle shows contact with bacterial cell *via* electrostatic interaction, van der waals forces, receptor-ligand, and hydrophobic interactions **(Choi et al., 2017)**. Nanoparticle interacts with the basic component of cell and causes oxidative stress, permeability, and gene expression changes electrolyte balance disorders, protein deactivation, and enzyme inhibition, and other diverse alterations **(Yang et al., 2009).**

Silver nanoparticles of antibiotics like penicillin G, amoxicillin, erythromycin, and vancomycin amplified the antibacterial activity and reduced the formation of biofilm in bacteria by different methods like change in the permeability of the membrane, cell wall, and cytoplasm.

Nanoparticles of Au, Mg, NO, ZnO, CuO, Fe3O4, and YF are also used in the antibacterial activity **(Wang et al., 2017).**

Phytonanotechnology also is widely used because of its rapid, eco-friendly, non-toxic, and cost-effective protocols of synthesis process without the use of energy, temperature, toxic chemicals, or high pressure. The plant extract is mixed with a metal precursor solution at room temperature and particular pH, which is the process of formation of the plant nanoparticles **(Mohamad, et al., 2014).**

3. **Liposomes as drug delivery vehicles**

Liposomes are spherical vesicles which consist of one or more lipid layers, of a particular size of approx 30nm to several micrometers. The liposomes surround the aqueous space and are used as a target drug delivery system. There are different types of liposomes like BBLs (biomineral-binding liposomes), LLSs (liposome loaded scaffolds), SSLs (solid supported liposomes) which work in drug delivery like Vancomycin, gentamicin, Triclosan, chlorhexidine, Benzylpenicillin G, Amikacin, Tobramycin, Meropenem ,etc **(Poerio et al., 2017).**

Nanoparticles form of liposomes like (liposomal NPs, solid lipid (SL) NPs, polymer-based NPs, inorganic nano drug carriers, terpenoid-based NPs, and dendrimer NPs) are effective in combating microbial resistance by reducing the mechanism of resistance and work as a carrier of antibiotics **(Ranghar et al., 2013).**

Furthermore, the release of antibiotics can be controlled by maintaining optimum concentration at the infection site for a prolonged time, which reduces the frequency of medication along with the inhibitory effect on cell growth **(Liu et al., 2016).** Importantly, on the nanoparticle, the different drug combinations can also be carried to inhibit MDR microbes**.**

Other strategies by the use of Molecular advancement techniques

A. Antisense agents

Antisense peptides are nucleic acid, which is gene-specific and help in the inhibition of bacteria with a specific sequence. Antimicrobial resistance gene with antisense agents is used as a molecular method to reduce the expression of resistance and reestablished sensitivity in bacteria **(Ali et al., 2018).**

The sequential use of two or more drugs alternative or in combinations may increase the resistance to both the drugs. As when one drug sensitizes the bacteria for the second drug while minimizing the cross-resistance, the resistance to one drug confers resistance to the second drug. Pair of synergistic antibiotics is more efficient than the cumulative efficiency of each antibiotic when used alone, as their dual actions are thought to be more difficult to overcome. Unfortunately, it is difficult to find synergistic pairs because it requires screening a large number of drug combinations **(Wambaugh et al., 2017).**

So with the use of specifically designed antisense oligonucleotide, the resistant bacteria can be re-sensitized. The oligonucleotide is a peptide-conjugated phosphorodiamidate morpholino

oligomer (PPMO), act as an antisense mRNA is a translational inhibitor and is designed to target the mRNAs which encode resistant genes. The study concludes that various PPMOs that are effective targets the drug efflux pump, and treatment by this PPMO can increase the antibiotic efficacy of around 2 to 40 fold **(Richardson, 2017)**

B. Antimicrobial peptides

Bacteriocins and defensins are cationic and amphiphilic peptide consists of 20-50 amino acids. The interactions of these peptides with negatively charged bacterial membrane leads to the death of the cell because of the formation of pores in the transmembrane which causes leakage of cellular solutes. Genetic determinants for the production of bacteriocin are located on a mobile genetic element. Most of the bacteriocins are identified from *E. coli* and other Enterobacteria. Bacteriocins inhibit microbes like *E. coli via,* inhibition of cell wall biosynthesis, *Clostridium difficile,* and other resistant bacteria like MRSA, VRE **(Gordya et al., 2017)**. Defensins are a group of AMPs that are effective against Gram-positive bacteria. Defensins contain a-helix/b-sheet elements coordinated by three disulfide bridges **(de Leeuw et al., 2010)**

C. CRISPR CAS

With the expansion of knowledge and engineering capabilities, studies have now pushed toward tuning gene regulation and the development of new methods to combat antimicrobial resistance. Thus use of clustered regularly interspaced short palindromic repeats (CRISPR) system is used to re-sensitized bacteria for antibiotics **(Singh, 2015).** As genes which codes resistance are known so, current research focuses on target that genes of bacteria which are essential for their survival **(Ali et al., 2018)**. CRISPR is a technique use gRNA and Cas 9 protein modified sequence, as a technique is an adaptive immune mechanism that has the ability to cleave foreign DNA. There are three types of CRISPR-Cas9 systems, type I cleave and degrade DNA, type II cleave DNA and type III, cleave DNA, and RNA. The study shows that the technique can be used in human therapy and for targeting AMR. The year 2002 study revealed that CRISPR loci able to transcribe small RNAs and cas genes identify as part of the same family of the CRISPR loci **(Gomaa et al., 2014).**

One study revealed that the use of CRISPR- Cas 9 system to ESBL (extended-spectrum β-lactamase) in *E.coli* target the conserved sequence in ESBL mutants to resensitized the bacteria against antibiotics. It seems that the target sequenced used to resensitize multidrug-resistant cell in which resistance is mediated by those genes that are not the target of the CRISPR/Cas9 system, but by genes that are present on the same plasmid as target genes. About 99% of the cells were killed which was related to ESBL plasmid, so the attention given to the CRISPR technique which has the potential against AMR **(Kim et al., 2016).** The technique also used to remove resistant genes from AMR bacteria by gene editing thus reduce the number of resistant organisms and also decrease their ability for human infection.

Different approaches can also be used in genomics for the identification of new bacterial targets and new perspectives of targeting bacterial pathogens. Other strategies include like designing of

a molecule that blocks the bacteria attachment to its target site and also targets bacterial virulence factor also contribute to the production of antibodies which inactivate bacteria, these seem to be an acceptable option to tackle the drug resistance problem **(Sultan et al., 2018).**

Conclusion

A bacterial infection keeps on being one of the main causes of morbidity and mortality around the world. The revolutions in antibiotics research, rescued several lives from infectious diseases. New categories of antibiotics were discovered subsequently for half a century, but they show resistance soon after their introductory period. Different articles address various timely issues identified with antibiotic resistance mechanisms. The discovery of new antibiotics, as well as new strategies to increase the life of existing antibiotics, is important to fight against the ever-increasing antimicrobial resistance. Bacteria, however, possess a large diversity of genes that permit them, sooner or later, to counteract the action of newly invented antibiotics.
The excessive and imprudent use of antibiotics, widespread spreading of resistant determinants as part of MGEs has increased the rate of resistance development in bacteria. On the other hand, all renowned antibiotic classes have earned notable resistance thus monotherapy approaches have become limited in the landscape of MDR pathogens. By molecular studies, different mechanisms in microbes are identified to attain the antimicrobial resistance. This adverse condition of antimicrobial resistance demands for the renewal for the development of new and efficient drugs to treat the various deadly infections and natural plant based drugs is very efficient method of treating resistant microbes and its combination strategies is considered as great technology for inhibition of MDR strains. Other Molecular methods are also very efficient to solve this global problem. Intending to decrease the threat of antibiotic resistance, it seems essential for everybody to have some basic knowledge about the systems to ensure optimal use of antibiotics from the surrounding milieu, so the development of antibiotic-resistant superbugs will become slow.

References

1. Fleming A. (1929). On the Antibacterial Action of Cultures of a Penicillium, with Special Reference to their Use in the Isolation of B. influenzæ. *British journal of experimental pathology*, *10*(3), 226–236.

2. Pillai, S.K., Moellering, R.C. and Eliopoulos, G.M. (2005) Antimicrobial Combinations. In: Lorian, V., Ed., Antibiotics in Laboratory Medicine, 5th Edition, the Lippincott Williams & Wilkins Co., Philadelphia, 365-440.
3. Mohamad NAN ,Arham NA ,Jai J ,Hadi A .(2014) Plant extract as reducing agent in synthesis of metallic nanoparticles: a review. Adv Mater Res;832:350–5 .

4. Abraham, E. P., Chain, E., Fletcher, C. M., Gardner, A. D., Heatley, N. G., Jennings, M. A., & Florey, H. W. (1941). FURTHER OBSERVATIONS ON PENICILLIN. *The Lancet*, *238*(6155), 177–189. https://doi.org/10.1016/S0140-6736(00)72122-2
5. Aiyegoro, O., Afolayan, A., & Okoh, A. (2009). In vitro antibacterial activities of crude extracts of the leaves of Helichrysum longifolium in combination with selected antibiotics. *African Journal of Pharmacy and Pharmacology*. https://www.semanticscholar.org/paper/In-vitro-antibacterial-activities-of-crude-extracts-Aiyegoro-Afolayan/52889b38fa86914a57b38f438fddae328d2ef906
6. Ali, J., Rafiq, Q. A., & Ratcliffe, E. (2018). Antimicrobial resistance mechanisms and potential synthetic treatments. *Future Science OA*, *4*(4), FSO290. https://doi.org/10.4155/fsoa-2017-0109
7. Alonso, M., Marín, M., Iglesias, C., Cercenado, E., Bouza, E., & García De Viedma, D. (2014). Rapid identification of linezolid resistance in Enterococcus spp. Based on high-resolution melting analysis. *Journal of Microbiological Methods*, *98*, 41–43. https://doi.org/10.1016/j.mimet.2013.12.013
8. Anand, U., Jacobo-Herrera, N., Altemimi, A., & Lakhssassi, N. (2019). A Comprehensive Review on Medicinal Plants as Antimicrobial Therapeutics: Potential Avenues of Biocompatible Drug Discovery. *Metabolites*, *9*(11). https://doi.org/10.3390/metabo9110258
9. Andersson, D. I., & Hughes, D. (2011). Persistence of antibiotic resistance in bacterial populations. *FEMS Microbiology Reviews*, *35*(5), 901–911. https://doi.org/10.1111/j.1574-6976.2011.00289.x
10. Aslam, B., Wang, W., Arshad, M. I., Khurshid, M., Muzammil, S., Rasool, M. H., Nisar, M. A., Alvi, R. F., Aslam, M. A., Qamar, M. U., Salamat, M. K. F., & Baloch, Z. (2018). Antibiotic resistance: A rundown of a global crisis. *Infection and Drug Resistance*, *11*, 1645–1658. https://doi.org/10.2147/IDR.S173867
11. Assob, J. C. N., Kamga, H. L. F., Nsagha, D. S., Njunda, A. L., Nde, P. F., Asongalem, E. A., Njouendou, A. J., Sandjon, B., & Penlap, V. B. (2011). Antimicrobial and toxicological activities of five medicinal plant species from Cameroon traditional medicine. *BMC Complementary and Alternative Medicine*, *11*, 70. https://doi.org/10.1186/1472-6882-11-70
12. Baldwin, P. R., Reeves, A. Z., Powell, K. R., Napier, R. J., Swimm, A. I., Sun, A., Giesler, K., Bommarius, B., Shinnick, T. M., Snyder, J. P., Liotta, D. C., & Kalman, D. (2015). Monocarbonyl analogs of curcumin inhibit growth of antibiotic sensitive and resistant strains of Mycobacterium tuberculosis. *European Journal of Medicinal Chemistry*, *92*, 693–699. https://doi.org/10.1016/j.ejmech.2015.01.020
13. Bayda, S., Hadla, M., Palazzolo, S., Riello, P., Corona, G., Toffoli, G., & Rizzolio, F. (2018). Inorganic Nanoparticles for Cancer Therapy: A Transition from Lab to Clinic. *Current Medicinal Chemistry*, *25*(34), 4269–4303. https://doi.org/10.2174/0929867325666171229141156
14. Blair, J. M. A., Webber, M. A., Baylay, A. J., Ogbolu, D. O., & Piddock, L. J. V. (2015). Molecular mechanisms of antibiotic resistance. *Nature Reviews Microbiology*, *13*(1), 42–51. https://doi.org/10.1038/nrmicro3380
15. Buchmann, D., Schultze, N., Borchardt, J., Böttcher, I., Schaufler, K., & Guenther, S. (2022). Synergistic antimicrobial activities of epigallocatechin gallate, myricetin, daidzein, gallic acid, epicatechin, 3-hydroxy-6-methoxyflavone and genistein combined

with antibiotics against ESKAPE pathogens. *Journal of Applied Microbiology*, *132*(2), 949–963. https://doi.org/10.1111/jam.15253
16. Chandra, H., Bishnoi, P., Yadav, A., Patni, B., Mishra, A., & Nautiyal, A. (2017). Antimicrobial Resistance and the Alternative Resources with Special Emphasis on Plant-Based Antimicrobials—A Review. *Plants*, *6*(4), 16. https://doi.org/10.3390/plants6020016
17. Chin, L. W., Cheng, Y.-W., Lin, S.-S., Lai, Y.-Y., Lin, L.-Y., Chou, M.-Y., Chou, M.-C., & Yang, C.-C. (2010). Anti-herpes simplex virus effects of berberine from Coptidis rhizoma, a major component of a Chinese herbal medicine, Ching-Wei-San. *Archives of Virology*, *155*(12), 1933–1941. https://doi.org/10.1007/s00705-010-0779-9
18. Choi, H.-J., Pammi, S. V. N., Park, B.-J., Eom, J.-H., An, H., Kim, H. Y., Kim, M., Seol, D., Kim, Y., & Yoon, S.-G. (2017). Resistance against water and acid water (pH = 4.0) via Al-doped ZnO thin films for environmentally friendly glass panels. *Journal of Alloys and Compounds*, *719*, 271–280. https://doi.org/10.1016/j.jallcom.2017.05.190
19. Chou, T.-C. (2010). Drug combination studies and their synergy quantification using the Chou-Talalay method. *Cancer Research*, *70*(2), 440–446. https://doi.org/10.1158/0008-5472.CAN-09-1947
20. Dadgar, T., M, A., A, S., M, M., H, B., Moradi, A., M, B., & Ghaemi, E. A. (2006). Antibacterial Activity of Certain Iranian Medicinal Plants Against Methicillin-Resistant and Sensitive Staphylococcus aureus. *Asian Journal of Plant Sciences*, *5*. https://doi.org/10.3923/ajps.2006.861.866
21. Davies, J., & Davies, D. (2010a). Origins and Evolution of Antibiotic Resistance. *Microbiology and Molecular Biology Reviews : MMBR*, *74*(3), 417–433. https://doi.org/10.1128/MMBR.00016-10
22. Davies, J., & Davies, D. (2010b). Origins and Evolution of Antibiotic Resistance. *Microbiology and Molecular Biology Reviews : MMBR*, *74*(3), 417–433. https://doi.org/10.1128/MMBR.00016-10
23. De Filippis, L. F. (2016). Plant secondary metabolites: From molecular biology to health products. In M. M. Azooz & P. Ahmad (Eds.), *Plant-Environment Interaction* (1st ed., pp. 263–299). Wiley. https://doi.org/10.1002/9781119081005.ch15
24. de Leeuw, E., Li, C., Zeng, P., Li, C., Diepeveen-de Buin, M., Lu, W.-Y., Breukink, E., & Lu, W. (2010). Functional Interaction of Human Neutrophil Peptide-1 with the cell wall precursor Lipid II. *FEBS Letters*, *584*(8), 1543–1548. https://doi.org/10.1016/j.febslet.2010.03.004
25. El-Halfawy, O. M., Klett, J., Ingram, R. J., Loutet, S. A., Murphy, M. E. P., Martín-Santamaría, S., & Valvano, M. A. (2017). Antibiotic Capture by Bacterial Lipocalins Uncovers an Extracellular Mechanism of Intrinsic Antibiotic Resistance. *MBio*, *8*(2), e00225-17. https://doi.org/10.1128/mBio.00225-17
26. Embley, T. M., & Stackebrandt, E. (1994). The molecular phylogeny and systematics of the actinomycetes. *Annual Review of Microbiology*, *48*, 257–289. https://doi.org/10.1146/annurev.mi.48.100194.001353
27. Fernandes, P., & Martens, E. (2017). Antibiotics in late clinical development. *Biochemical Pharmacology*, *133*, 152–163. https://doi.org/10.1016/j.bcp.2016.09.025
28. Fleming, A. (1929). On the Antibacterial Action of Cultures of a Penicillium, with Special Reference to their Use in the Isolation of B. influenzæ. *British Journal of Experimental Pathology*, *10*(3), 226–236.

29. Frost, W. D. (2018). *The Antagonism Exhibited by Certain Saprophytic Bacteria Against the Bacillus Typhosus Gaffky*. Forgotten Books.
30. Gallo, V., Mastrorilli, P., Cafagna, I., Nitti, G., Latronico, M., Longobardi, F., Minoja, A., Napoli, C., Romito, V., Schäfer, H., Schütz, B., & Spraul, M. (2014). Effects of agronomical practices on chemical composition of table grapes evaluated by NMR spectroscopy. *Journal of Food Composition and Analysis*, *35*. https://doi.org/10.1016/j.jfca.2014.04.004
31. García, A., Bocanegra-García, V., Palma-Nicolás, J. P., & Rivera, G. (2012). Recent advances in antitubercular natural products. *European Journal of Medicinal Chemistry*, *49*, 1–23. https://doi.org/10.1016/j.ejmech.2011.12.029
32. Garner, M. J., Carson, C., Lingohr, E. J., Fazil, A., Edge, V. L., & Trumble Waddell, J. (2015). An assessment of antimicrobial resistant disease threats in Canada. *PloS One*, *10*(4), e0125155. https://doi.org/10.1371/journal.pone.0125155
33. Gelpi, A., Gilbertson, A., & Tucker, J. D. (2015). Magic bullet: Paul Ehrlich, Salvarsan and the birth of venereology. *Sexually Transmitted Infections*, *91*(1), 68–69. https://doi.org/10.1136/sextrans-2014-051779
34. Gomaa, A. A., Klumpe, H. E., Luo, M. L., Selle, K., Barrangou, R., & Beisel, C. L. (2014). Programmable Removal of Bacterial Strains by Use of Genome-Targeting CRISPR-Cas Systems. *MBio*, *5*(1), e00928-13. https://doi.org/10.1128/mBio.00928-13
35. Gordya, N., Yakovlev, A., Kruglikova, A., Tulin, D., Potolitsina, E., Suborova, T., Bordo, D., Rosano, C., & Chernysh, S. (2017). Natural antimicrobial peptide complexes in the fighting of antibiotic resistant biofilms: Calliphora vicina medicinal maggots. *PLOS ONE*, *12*(3), e0173559. https://doi.org/10.1371/journal.pone.0173559
36. Gu, B., Kelesidis, T., Tsiodras, S., Hindler, J., & Humphries, R. M. (2013). The emerging problem of linezolid-resistant *Staphylococcus*. *Journal of Antimicrobial Chemotherapy*, *68*(1), 4–11. https://doi.org/10.1093/jac/dks354
37. Gupta, P. D., & Birdi, T. J. (2017). Development of botanicals to combat antibiotic resistance. *Journal of Ayurveda and Integrative Medicine*, *8*(4), 266–275. https://doi.org/10.1016/j.jaim.2017.05.004
38. Hutchings, M. I., Truman, A. W., & Wilkinson, B. (2019). Antibiotics: Past, present and future. *Current Opinion in Microbiology*, *51*, 72–80. https://doi.org/10.1016/j.mib.2019.10.008
39. Jang, E.-J., Cha, S.-M., Choi, S.-M., & Cha, J.-D. (2014). Combination effects of baicalein with antibiotics against oral pathogens. *Archives of Oral Biology*, *59*(11), 1233–1241. https://doi.org/10.1016/j.archoralbio.2014.07.008
40. Kessler, A., & Kalske, A. (2018). Plant Secondary Metabolite Diversity and Species Interactions. *Annual Review of Ecology, Evolution, and Systematics*, *49*(1), 115–138. https://doi.org/10.1146/annurev-ecolsys-110617-062406
41. Khameneh, B., Iranshahy, M., Soheili, V., & Fazly Bazzaz, B. S. (2019). Review on plant antimicrobials: A mechanistic viewpoint. *Antimicrobial Resistance and Infection Control*, *8*, 118. https://doi.org/10.1186/s13756-019-0559-6
42. Kim, J.-S., Cho, D.-H., Park, M., Chung, W.-J., Shin, D., Ko, K. S., & Kweon, D.-H. (2016). CRISPR/Cas9-Mediated Re-Sensitization of Antibiotic-Resistant Escherichia coli Harboring Extended-Spectrum ββ-Lactamases. *Journal of Microbiology and Biotechnology*, *26*(2), 394–401. https://doi.org/10.4014/jmb.1508.08080

43. Kohler, I., Verhoeven, A., Derks, R. J., & Giera, M. (2016). Analytical pitfalls and challenges in clinical metabolomics. *Bioanalysis*, *8*(14), 1509–1532. https://doi.org/10.4155/bio-2016-0090
44. Kumar, S., Ingle, H., Prasad, D. V. R., & Kumar, H. (2013). Recognition of bacterial infection by innate immune sensors. *Critical Reviews in Microbiology*, *39*(3), 229–246. https://doi.org/10.3109/1040841X.2012.706249
45. Liu, J. L., Liu, J. L., Zhang, W. J., Li, X. D., Yang, N., Pan, W. S., Kong, J., & Zhang, J. S., & 34., L., J. L. ,. Zhang, W. J. ,. Li, X. D. ,. Yang, N. ,. Pan, W. S. ,. Kong, J. ,. &. Zhang, J. S. (2016). Sustained-release genistein from nanostructured lipid carrier suppresses human lens epithelial cell growth. *International Journal of Ophthalmology*. https://doi.org/10.18240/ijo.2016.05.01
46. Manson, J. M., Hancock, L. E., & Gilmore, M. S. (2010). Mechanism of chromosomal transfer of *Enterococcus faecalis* pathogenicity island, capsule, antimicrobial resistance, and other traits. *Proceedings of the National Academy of Sciences*, *107*(27), 12269–12274. https://doi.org/10.1073/pnas.1000139107
47. Marsh, K., IJzerman, M., Thokala, P., Baltussen, R., Boysen, M., Kaló, Z., Lönngren, T., Mussen, F., Peacock, S., Watkins, J., Devlin, N., & ISPOR Task Force. (2016). Multiple Criteria Decision Analysis for Health Care Decision Making--Emerging Good Practices: Report 2 of the ISPOR MCDA Emerging Good Practices Task Force. *Value in Health: The Journal of the International Society for Pharmacoeconomics and Outcomes Research*, *19*(2), 125–137. https://doi.org/10.1016/j.jval.2015.12.016
48. Mccreath, S. B., & Delgoda, R. (2017). *Pharmacognosy: Fundamentals, Applications and Strategies*. Academic Press.
49. Moussaoui, F., & Alaoui, T. (2015). Evaluation of antibacterial activity and synergistic effect between antibiotic and the essential oils of some medicinal plants. *Asian Pacific Journal of Tropical Biomedicine*, *6*. https://doi.org/10.1016/j.apjtb.2015.09.024
50. Munita, J. M., & Arias, C. A. (2016). Mechanisms of Antibiotic Resistance. *Microbiology Spectrum*, *4*(2), 4.2.15. https://doi.org/10.1128/microbiolspec.VMBF-0016-2015
51. O'Neill, J. (2014). Antimicrobial resistance: Tackling a crisis for the health and wealth of nations. *Rev. Antimicrob. Resist.* https://cir.nii.ac.jp/crid/1370857593729357568
52. Pendleton, J. N., Gorman, S. P., & Gilmore, B. F. (2013). Clinical relevance of the ESKAPE pathogens. *Expert Review of Anti-Infective Therapy*, *11*(3), 297–308. https://doi.org/10.1586/eri.13.12
53. Peng, F. Y., Hu, Z., & Yang, R.-C. (2015). Genome-Wide Comparative Analysis of Flowering-Related Genes in Arabidopsis, Wheat, and Barley. *International Journal of Plant Genomics*, *2015*, e874361. https://doi.org/10.1155/2015/874361
54. Peterson, E., & Kaur, P. (2018). Antibiotic Resistance Mechanisms in Bacteria: Relationships Between Resistance Determinants of Antibiotic Producers, Environmental Bacteria, and Clinical Pathogens. *Frontiers in Microbiology*, *9*, 2928. https://doi.org/10.3389/fmicb.2018.02928
55. Poerio, N., Bugli, F., Taus, F., Santucci, M. B., Rodolfo, C., Cecconi, F., Torelli, R., Varone, F., Inchingolo, R., Majo, F., Lucidi, V., Mariotti, S., Nisini, R., Sanguinetti, M., & Fraziano, M. (2017). Liposomes loaded with bioactive lipids enhance antibacterial innate immunity irrespective of drug resistance. *Scientific Reports*, *7*(1), 45120. https://doi.org/10.1038/srep45120

56. Ranghar, S., Sirohi, P., Verma, P., & Agarwal, V. (2013). Nanoparticle-based drug delivery systems: Promising approaches against infections. *Brazilian Archives of Biology and Technology*, *57*(2), 209–222. https://doi.org/10.1590/S1516-89132013005000011
57. Richardson, L. A. (2017). Understanding and overcoming antibiotic resistance. *PLOS Biology*, *15*(8), e2003775. https://doi.org/10.1371/journal.pbio.2003775
58. Rosato, A., Vitali, C., De Laurentis, N., Armenise, D., & Antonietta Milillo, M. (2007). Antibacterial effect of some essential oils administered alone or in combination with Norfloxacin. *Phytomedicine: International Journal of Phytotherapy and Phytopharmacology*, *14*(11), 727–732. https://doi.org/10.1016/j.phymed.2007.01.005
59. Ruddaraju, L., Pammi, S. V. N., Sankar, G., Padavala, V., & Kolapalli, V. (2019). A review on anti-bacterials to combat resistance: From ancient era of plants and metals to present and future perspectives of green nano technological combinations. *Asian Journal of Pharmaceutical Sciences*, *15*. https://doi.org/10.1016/j.ajps.2019.03.002
60. Russell, A. (2003). Biocide use and antibiotic resistance: The relevance of laboratory findings to clinical and environmental situations. *The Lancet Infectious Diseases*, *3*(12), 794–803. https://doi.org/10.1016/S1473-3099(03)00833-8
61. Savoia, D. (2012). Plant-derived antimicrobial compounds: Alternatives to antibiotics. *Future Microbiology*, *7*(8), 979–990. https://doi.org/10.2217/fmb.12.68
62. Sher, A. (2004). ANTIMICROBIAL ACTIVITY OF NATURAL PRODUCTS FROM MEDICINAL PLANTS. *Gomal Journal of Medical Sciences*. https://www.semanticscholar.org/paper/ANTIMICROBIAL-ACTIVITY-OF-NATURAL-PRODUCTS-FROM-Sher/6dcffb55988327676bf768217d10cc4ca15f2db3
63. Singh, R. P. (2015). Attenuation of quorum sensing-mediated virulence in Gram-negative pathogenic bacteria: Implications for the post-antibiotic era. *MedChemComm*, *6*(2), 259–272. https://doi.org/10.1039/C4MD00363B
64. Stanton, T. B. (2013). A call for antibiotic alternatives research. *Trends in Microbiology*, *21*(3), 111–113. https://doi.org/10.1016/j.tim.2012.11.002
65. Strachan, C. R., & Davies, J. (2017). The Whys and Wherefores of Antibiotic Resistance. *Cold Spring Harbor Perspectives in Medicine*, *7*(2), a025171. https://doi.org/10.1101/cshperspect.a025171
66. Sultan, I., Rahman, S., Jan, A. T., Siddiqui, M. T., Mondal, A. H., & Haq, Q. M. R. (2018). Antibiotics, Resistome and Resistance Mechanisms: A Bacterial Perspective. *Frontiers in Microbiology*, *9*, 2066. https://doi.org/10.3389/fmicb.2018.02066
67. Tacconelli, E., Carrara, E., Savoldi, A., Harbarth, S., Mendelson, M., Monnet, D. L., Pulcini, C., Kahlmeter, G., Kluytmans, J., Carmeli, Y., Ouellette, M., Outterson, K., Patel, J., Cavaleri, M., Cox, E. M., Houchens, C. R., Grayson, M. L., Hansen, P., Singh, N., … WHO Pathogens Priority List Working Group. (2018). Discovery, research, and development of new antibiotics: The WHO priority list of antibiotic-resistant bacteria and tuberculosis. *The Lancet. Infectious Diseases*, *18*(3), 318–327. https://doi.org/10.1016/S1473-3099(17)30753-3
68. Tiwari, P., Kaur, M., & Kaur, H. (2011). *Phytochemical screening and Extraction: A Review*. https://www.semanticscholar.org/paper/Phytochemical-screening-and-Extraction%3A-A-Review-Tiwari-Kaur/979e9b8ddd64c0251740bd8ff2f65f3c9a1b3408
69. Vaou, N., Stavropoulou, E., Voidarou, C., Tsigalou, C., & Bezirtzoglou, E. (2021). Towards Advances in Medicinal Plant Antimicrobial Activity: A Review Study on

Challenges and Future Perspectives. *Microorganisms*, *9*(10), 2041. https://doi.org/10.3390/microorganisms9102041

70. Wambaugh, M. A., Shakya, V. P. S., Lewis, A. J., Mulvey, M. A., & Brown, J. C. S. (2017). High-throughput identification and rational design of synergistic small-molecule pairs for combating and bypassing antibiotic resistance. *PLOS Biology*, *15*(6), e2001644. https://doi.org/10.1371/journal.pbio.2001644
71. Wang, L., Hu, C., & Shao, L. (2017). The antimicrobial activity of nanoparticles: Present situation and prospects for the future. *International Journal of Nanomedicine*, *Volume 12*, 1227–1249. https://doi.org/10.2147/IJN.S121956
72. *WHO | Global action plan on AMR*. (n.d.). WHO; World Health Organization. Retrieved May 19, 2021, from http://www.who.int/antimicrobial-resistance/global-action-plan/en/
73. Wink, M., Ashour, M. L., & El-Readi, M. Z. (2012). Secondary Metabolites from Plants Inhibiting ABC Transporters and Reversing Resistance of Cancer Cells and Microbes to Cytotoxic and Antimicrobial Agents. *Frontiers in Microbiology*, *3*, 130. https://doi.org/10.3389/fmicb.2012.00130
74. Yang, W., Shen, C., Ji, Q., An, H., Wang, J., Liu, Q., & Zhang, Z. (2009). Food storage material silver nanoparticles interfere with DNA replication fidelity and bind with DNA. *Nanotechnology*, *20*(8), 085102. https://doi.org/10.1088/0957-4484/20/8/085102