Current Research in Microbiology

Chapter 1

Development of Drug Resistant in Microbes and Therapeutic Antimicrobial Activity of some Medicinal Plants and their MetaboLites: Current Status

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Abstract

There has never been much requirement of broad, well-researched information about herbs' as prospective to fight against infection. Pathogenic microbes have frequently been considered as a global threat due to development of drug resistance that significantly restricts the efficiency of currently used antimicrobial drugs. Therefore, Researchers had work continuously to find some breakthrough regarding treatment of drug resistant microbes and they added to the growing body of knowledge about herbs. Herbs are used

secondary metabolites with different a biological function that produce wide range of activity to enhance immune system of human body and inhibits the growth of microbes, can serve as source for the synthesis of new antimicrobial drugs, especially against antibiotic resistant bacteria.

Keywords: Drug resistant; Antimicrobial activity; Medicinal plants; Antimicrobial therapy

since long in the alternative therapy to treat various infectious disease without knowing there mechanism of action. Plants contain large number of different

Introduction

Pathogenic microbes have frequently been considered as a common global problem posing major threat to public health concerns due to the emergence and spread of multidrug drug resistant (MDR) strains of pathogenic microbes [1]. The harmful microorganisms can be controlled with drugs however, inappropriate usage of antimicrobial agents responsible for development of antibiotic resistance and the global emergence of MDR that significantly limiting the effectiveness of currently used drugs and threatened the failure of current therapy in the treatment of infections [2,3], making the treatment difficult, costly, or even impossible. Further, chances of reemergence of previously benign diseases and a substantial spread of chronic infections are increased that may resulted in increase in morbidity and mortality each year [4]. Therefore, there is a pressing need to look for substances from other sources with proven antimicrobial activity against drug resistant phenotypes. Among the potential sources of new agents, plants have long been studied, because plants contain large number of different bioactive constituents with different biological activities that produce a definite physiological action on the human body, can serve as source for the synthesis of new antimicrobial drugs, especially against antibiotic resistant bacteria [5,6]. According to World Health Organization (WHO) more than 80% of the world's population relies on use traditional and botanical medicines for their primary healthcare needs and consider them to be a normal part of primary healthcare [7]. A large number of medicinal plants have been documented as important resources of natural antimicrobial compounds that have been used as traditional medicines in India/World, for infectious diseases treatment [8].

2. What is Multidrug Resistant (MDR)?

Antimicrobial resistance (AMR) is not a new phenomenon. It is the ability of a microbe to resist the effects of antimicrobial compounds produced by other microorganisms previously used to treat them. "Antibiotic resistance", is the most specific term used only for bacteria becoming resistant to antibiotics. Treatment of resistant microbes is not easy, it requires some alternative therapy or higher doses or both, that may be highly expensive and toxic too. Microbes resistant to multiple antimicrobials agents are called multidrug resistant (MDR) or sometimes superbugs.

2.1 Antimicrobial resistance and global concern

Antimicrobial resistance is increases very sharply and it is estimated that several million deaths every year. In USA alone at least 2 million people come in contact with bacteria that are resistant to antibiotics and as a result thousands of people die [9]. So, there is need of global awareness and worldwide collective action to deal with the threat include proposal for treaties on AMR.

2.2. Cause and spread of MDR

Development of drugs for the treatment of microbes and their extensive clinical use offered another reason to encourage further evolution. There are various factors like inappropriate/ misuse of antimicrobial drugs and noncompliance with the suggested treatment course by patients, are mainly responsible to accelerate the evolution of drug resistance **(Figure1)**.

Spread of antimicrobial resistance occur are due to releasing of huge amount of antimicrobial agent into the surrounding environment through manufacturing unit of pharmaceutical industry, during wastewater treatment, and also by use of soap and other products with antibacterial component. Further, any use of contaminated water and come in contact with infected workers [10].

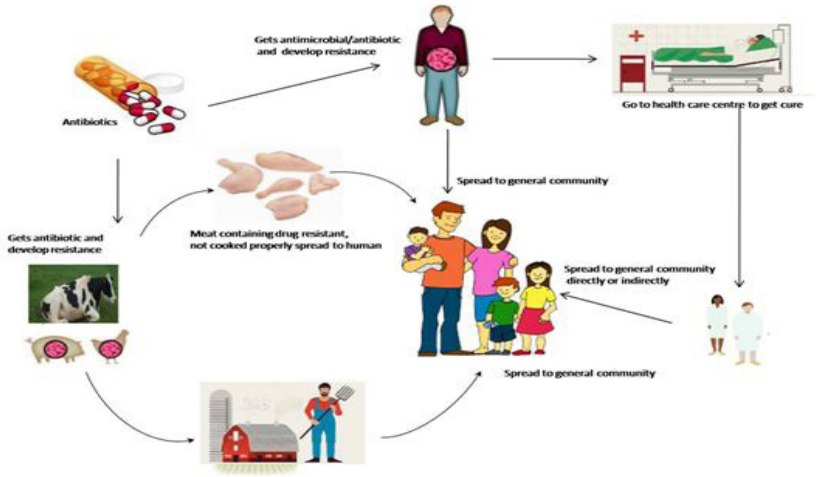

Figure 1: Spread of drug resistant microbes in general population

2.3. Antimicrobial drugs and their target

Some commonly used antimicrobial agents and their target sites are presented in **Table 1**.

Table 1 : Common antimicrobial agents and their target sites.

Antimicrobial Agent	Target	Species
Fluoroquinolones	Topoisimerase	Salmonella., M. tuberculosis
Tetracylines	Ribosome	Staphylococcus spp.
Macrolides	Ribosome	Pseudomonas aeruginosa
Aminoglycosides	Ribosome	Neisseria Gonorrhoeae, Salmonella spp.
β-lactam	Penicilline binding proteins (PBPs)	Staphylococcus spp.
Rifamycins	RNA Polymerase	M. tuberculosis
Acyclovir	DNA Polymeerase	Herpesviruses
Amantadine and Rimantadine	M2 Proton Channel	influenza
Azole (fluconazole)	lanosterol14 α-demethy lase	candidaspecies and Cryptococcus neoformans
Echinocandin and Azole	1,3-β glucan synthase Lanosterol 14 α-demethylase	aspergillus Speecies

3. Methods of Acquiring Resistant Gene in Microbes

All classes of microbes can develop resistance **(Figure 2)**. Since, antimicrobial drugs are site specific targets and any slight change in the structure to those targets can hinder the drug binding, rendering the drug ineffective. It can appear spontaneously through some genetic mutation or more commonly with time it develops naturally.

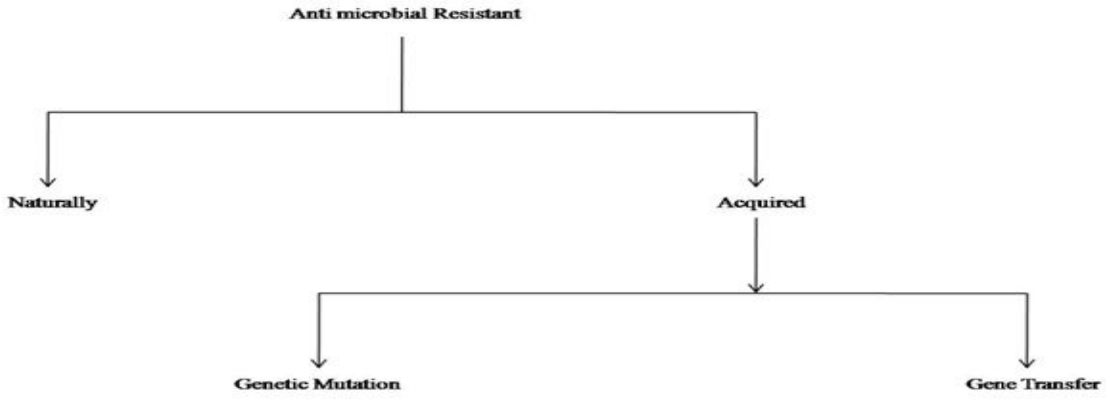

Figure 2 : Method of gaining antimicrobial resistance

3.1. Natural resistance

It is most common in certain types of bacteria. Such as, antibiotics that target cell wall synthesis are useless for Mycoplasma because of the lack of thick cell wall. In other example, Gram negative bacteria which are resistant to glycopeptide antibiotics like vancomycin, since the presence of small pores on outer covering that inhibit the antibiotic to move in from small pores. Further, genes those are resistant to antimicrobial agents are known as the environmental resistome and they can transfer from non-disease-causing microbes to other disease causing microbes, responsible for the considerable increase in number of clinically significant drug resistant microbial [11]. For Example, In the year 1952, Penicillin-resistant bacteria was found (preexisted), however penicillinase was detected in 1962 from the dormant endospores of *B. licheniformis* preserve since 1689 in British museum [12-14]. Penicillinase provides a kind of defense mechanism for bacteria in their natural environment, like in case of *S.aureus* which is a rich source of penicillinase, living with Trichophyton (penicillin-producing), however, this may be conditional.

3.2. Acquired resistance

In the presence of antimicrobial agents microbes either be suppressed or develop a resistance. If they are not resistant to antimicrobial agents naturally, they may either get resistance either by mutation (in case of virus/bacteria) or gene transfer (in case of bacteria). For Example-*Clostridium*, found in the bowels of members of the Franklin Expedition and after come in contact with this, they showed resistance to cefoxitin and clindamycin. The resistance was later found due to random mutation in the chromosomes of *Clostridium* strains [15].

Acyclovir is an antiviral drug used as preventive treatment against Herpes Simplex Virus (HSV) infections and encephalitis. In 1982, first and foremost clinical cases of acyclovir-resistant HSV were reported, soon after preliminary use of acyclovir [16-18]. Resistance of HSV to acyclovir is associated with mutations to viral thymidine kinase (TK) or DNA polymerase [19].

4. General Mechanism of Antimicrobial Resistance

Four general mechanisms described for the development of resistance in bacteria are summarized in **Figure 3** [20].

4.1. Efflux pump

Efflux pump is formed in some bacteria when antibiotic enters into the cell, which throws outside the bacterial by theses efflux pumps. This kind of resistance is seen in tetracycline resistant bacteria.

4.2. Target overproduction or enzymatic bypass

When an antimicrobial drug designs to act as an anti-metabolite, aiming to inhibit particular enzyme activity, then microbes protect themselves from antimicrobial drugs and develop resistance by either overproducing the target enzyme in a way that ample amount of antimicrobial-free enzyme to carry out the suitable enzymatic response or by developing a bypass mechanism that evades the need of target enzyme that requires for proper functioning of cell as found in sulfonamide resistance in *S. aureus, Pneumococci and E.Coli* etc.

Some sulfonamide resistant bacteria either over produce para-aminobenzoic acid (PABA), precursor for the synthesis of folic acid and nucleic acids or synthesize dihydropteroate synthetase (DHPS) enzyme that has low affinity for sulfonamides. Further may also use alternate pathway in folate metabolism (like mammalian cells start using preformed folic acid).

4.3. Alteration of target or target modification

Due to high targets specificity of the antimicrobial drugs any structural changes to those targets can prevent the binding of drug and making the drug ineffective. Any kind of genetic alteration in the active site of penicillin-binding proteins (PBPs), the binding target site of penicillins led the formation of resistance to multiple drugs as in case of β-lactam and methicillin (Methicillin resistant *S. aureus,* MRSA). Other reported mechanism found among bacterial species to protect themselves from antibiotics is binding of ribosomal protection proteins to the ribosomes of the bacterial cell. These proteins inhibit the binding of antibiotics to that of target cell's ribosomes (inhibit protein synthesis) through attaching themselves with the ribosome which in turn changes its conformational shape and protect bacterial cell. This

allows the ribosomes to continue synthesizing proteins essential to the cell while preventing antibiotics from binding to the ribosome to inhibit protein synthesis.

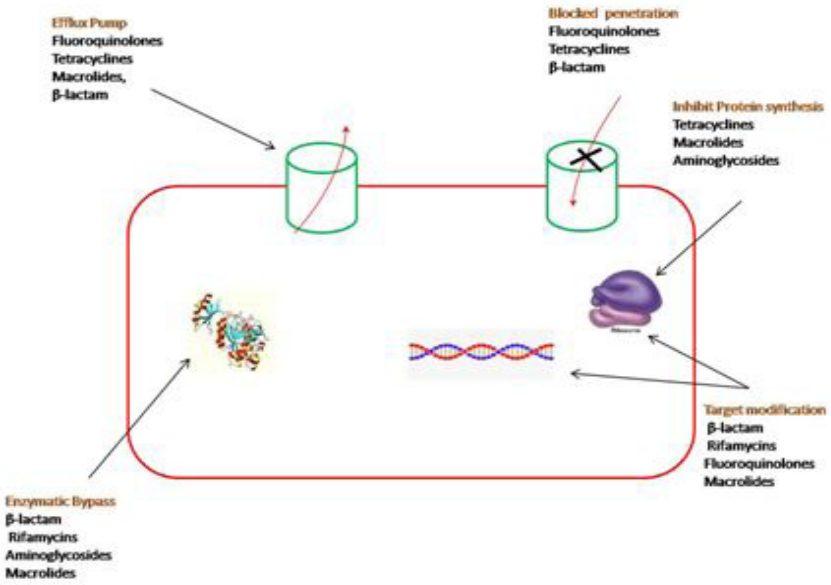

Figure 3 : Mechanism of antibiotic resistance

4.4. Target mimicry

Target mimicry is a newly find mechanism of drug resistance, involves the production and binding of proteins that mimic to the target of drugs, avert the drugs from binding to their target. For Example, *M.* tuberculosis produces regular pentapeptide repeats protein that binds fluoroquinolones because the structure of that protein appears to mimic the structure of DNA and thus this protein, confiscating them and preventing them from binding to DNA, providing *M.* tuberculosis resistance to fluoroquinolones **(Figure 4)** [21].

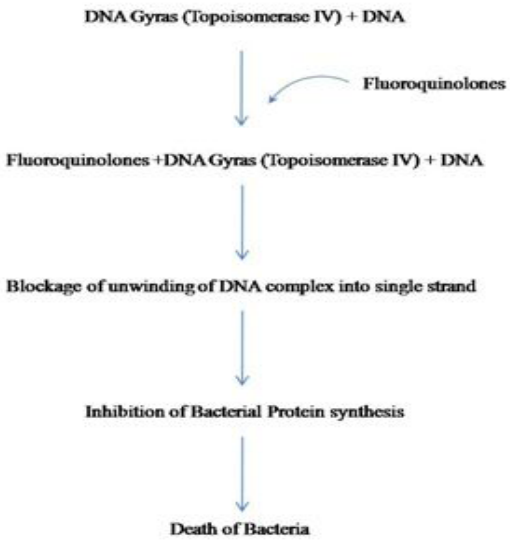

Figure 4 : Mechanism of action of fluoroquinolones

5. Preventive Measure

Antimicrobial resistance has become a grave and worldwide problem, collective effort have been done to address the threat including formation of global tracking system in order to identify and evaluate, trends in resistance on the worldwide level. In addition, international

treaty should be signed at global level to share information as well as necessary knowledge for evaluation and other changes made to fight or reverse antimicrobial resistance. Here, I am going to give some glimpse (not in detail) of preventive measures to minimize the drug resistance.

1. Duration of use of antimicrobial agent should be based on infection and other health problems

2. Development of a molecular method for detecting antimicrobial resistance genes

3. Antimicrobial stewardship teams should be in every health care facilities to encourage best possible use of antimicrobials

4. People should educate to optimal use of antimicrobials

5.1. Therapy

Over the past few decades, due to global emergence of MDR causes failure of current drugs against certain disease. Hence it essential to investigate newer drugs with lesser resistance. As a result, search for alternative medicine has begun and herbal drugs are one of them to treat infections because plant exhibits antimicrobial activity. Since ancient times, herbs are widely exploited in the traditional medicine and play major role in the prevention and cure of human diseases [22,23]. About 70% modern medicines developed were derived from natural source and they have been very successful to treat different ailments, especially in the areas of infectious disease [24]. Active constituents present in plants may provide a unique source of antimicrobial agents with possibly novel mechanisms of action [25,26].

Plants are rich source of several useful secondary metabolites such as tannins, terpenoids, alkaloids, flavonoids, glycosides, etc., which have been found in several in vitro or in vivo studies to have antimicrobial properties [27,28]. Here in this chapter we are going to talk about few plants and their metabolites having antimicrobial properties against drug resistance microbes.

5.1.1. *Cassia fistula*

Cassia fistula L., (Leguminosae), commonly known as Indian Labernum (also known as the Golden Shower), is native to India, Sri Lanka and various other countries like South Africa, Mexico, China, and Brazil [29]. *Cassia fistula* has been used since long as alternative therapy by tribal people to treat various diseases including ringworm and other fungal skin infections that support folkloric use of *Cassia fistula* as broad-spectrum antimicrobial agents [30,31]. Further, antimicrobial activity of alcoholic and aqueous extracts of stem bark of *Cassia fistula* was observed against antibiotic resistant (multi antibiotic resistant) *S. aureus* and it was found its antibacterial activity is more than that of reference antibiotic chloramphenicol [32]. In

confirmation to the previous study Chauhan et al has reported antimicrobial activity against some antibiotic resistant gram negative bacteria like *S. aureus* and *S, epidermidis* and also against some gram positive bacteria *E.coli, K.peumonia* [33]. Recently, antimicrobial activity of ethyl acetate and methanolic bark and leaf extract of *Cassia fistula* against ampicillin resistant strains of *E. coli*, *S. aureus* and fluconazole resistance fungi *C. albicans, C. neoformans* was confirmed by zone inhibition assay [34-37].

5.1.2. Azadirachta indica (Neem)

Neem is one of the most significant medicinal plants of India commonly known as "village pharmacy" as it most widely used as traditional medicine in India and has a wide biological activity. Every part of tree has its own medicinal property. Neem oil represses several disease causing bacteria that are multidrug resistance such as *S. aureus, S. typhosa*, and all strains of *M. tuberculosis* [38]. Further it also inhibits the growth of *V. cholerae*and *S. paratyphi* [39]. In addition, Neem has antiviral activity against HSV-1 infection into natural target cells which is reported to resistant to antiviral drug Acyclovir [40]. Due to its unique biological activity and precious source of distinctive natural products, it is now considered as wonder tree for the development of medicines against bacterial and viral infections.

5.1.3. *Curcuma longa* (Turmeric)

Curcuma longa, is a member of the Zingiberaceae family and use in alternative and traditional system of medicine in India and Chinese. The rhizomes, from *Curcuma longa*, reported to have antibacterial, anti-inflammatory, antineoplastic, and analgesic activities because they contains curcuminoids, biologically active constituent of *Curcuma longa* [41,42]. Real time polymerase chain (RT-PCR) analysis shows that curcuminoids isolated from *Curcuma longa* inhibited multidrug resistance-1 (MDR-1) gene expression [43]. Curcumin is the most important fraction of turmeric oil of *Curcuma longa* and responsible for the most of the biological activities of *Curcuma longa* having antibacterial activity against *S.albus, S. aureus* and *B. typhosus* [44,45].The results from synergistic study of curcumin in combination with some antibiotics, like ampicillin, norfloxacin and oxacillin against *S. aureus* strain proved that consumption of turmeric during treatment may help [45]. The synergistic effect of curcumin with ciprofloxacin against chloramphenicol, ampicillin, and trimethoprim (i.e. multidrug-resistant [MDR] strains) of *S. typhi and S. typhimurium* has also been reported and show its antagonistic activity against *S. typhi and S. typhimurium*in combination with ciprofloxacin [46,47].Antiviral drug resistance is one of the most common problems in medicine and use of natural plant product like curcumin and different derivatives against variety of viruses including influenza virus and HSV-1 in cell culture[48-50].

Alcoholic extract of turmeric confirmed antifungal activity against *C. neoformans* and *C. albicans*, which are antifungal resistant with minimum inhibitory concentration (MIC)

values of 128 and 256 µg/mL respectively [51]. Curcumin, chief constituent of turmeric also showed inhibitory effect on *C. neoformans* and *C. dubliniensis* through targeting the global suppressor thymidine uptake 1 (TUP1) [52,53]. Further, mixture of curcumin and ascorbic acid synergistically act against different strains of Candida much more significantly (5- to 10-fold reduction) than curcumin alone [54]. These synergistic effects showed that curcumin in combination with various fungicide materials can extensively enhance the efficiency of existing antifungal drugs. Keeping in view the vital role of *Curcuma longa* in inhibiting the activity of different microbes, can be said that it would be a boon in the field of drug development against microbes.

5.1.3. *Withania somnifera*

Withania somnifera (family Solanaceae) commonly known as Ashwagandha is an important medicinal herb that has been used in number of Indian herbal formulations since ancient times. Various studies have reported the antimicrobial activity *Withania somnifera* against various drug resistant microbes like *Enterococcus* (vancomycin-resistant), *E. coli* strain (tetracyclines, chloramphenicol, sulfonamides) *S. aureus* (MRSA) and *Candida* species (resistant to fluconazole) are associated with illness and are endemic in health care institutions, treatment with *Withania somnifera* leaf extract cause suppression of drug resistant microbes in a significant way [55-61]. In addition antiviral activity of *Withania somnifera* against HSV-1 was also reported [62].

5.1.4. *Viola yedoensis*

Viola yedoensis is a popular medicinal herb belonging to the family of Violaceae. It has been reported to have antimicrobial activity against drug resistant *Staphylococcus strain, E.coli* and *Salmonella* [63]. It also suppresses the HSV-1 multiplication in the human neuroblastoma SK-N-SH cell line. Cyclotides are macrocyclic plant peptides from Viola are shown to be effective in inhibiting influenza A and human immunodeficiency virus (HIV) replication [64,65].

5.1.5. *Andrographispaniculata*

Andrographis paniculata, commonly known as Anemone chinensi belongs to Acanthaceae family. *Andrographis paniculata* extensively used in Chinese, Arabic, Ayurveda, and Unani medicineas home remedy for treatment of various diseases in traditional system of medicine. It has potent antibacterial activity against MDR strain of *S. aureus, E. faecalis, S. Typhi, V. cholera* and *M. tuberculosis*[66,67].Antifungal activity of *Andrographis paniculata* was also documented against *C. lbicans, A. niger* and *A. lavus* [68].It also suppresses influenza A virus-induced RANTES secretion by human bronchial epithelial cells [69].

5.1.6. Cinnamomum

Cinnamomum is a evergreen aromatic shrubs and trees belonging to the family Lauraceae. Cinnamon is an aromatic oils obtained from their leaves and bark. It is used in traditional system of medicine in India for the treatment of various diseases. It has powerful antibacterial and antifungal activity against drug resistant strain of bacteria and fungi [70]. It also show some activity against yeast species [70-72].It also suppresses the multiplication and activity of influenza A and HSV-1 [73,74].

6. Conclusion

Antimicrobial drug resistance is a well-known feature in the management of persistent microbes. Due to this, interest in using natural plant based products and there metabolites for the development of antimicrobial agents to decrease the infection of microorganism is on rise. Investigation of the functions of different herbs and there secondary metabolites for treatment of many microbial diseases revealed that plants are effective antimicrobial agent and could be strong candidate for the development of new drug against drug resistant microbes. Hence these herbal drugs may find their means to treat bacterial or viral and fungal resistant infections as well as formulated in various antimicrobial formulations such as antiseptics, disinfectants, hand washes, dentifrices and other health care products.

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