Review Article

Endophytic Fungi - Alternative Therapy for Multi-Drug Resistant Pathogens - A Mini Review

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Received: 18-05-2019; **Revised:** 05-07-2019; **Accepted:** 22-07-2019.

ABSTRACT

Multidrug drug-resistant bacteria are becoming increasingly problematic predominantly in under developed countries. Methicillin-resistant *Staphylococcus aureus* (MRSA), Penicillin-resistant *Streptococcus pneumonia* and Vancomycin-resistant *Enterococcus faecium* are the major microorganisms that have seen to be a geometric rise in numbers. In order to overcome the increasing risk of drug-resistant strains of the human pathogen, there is a need in developing new antibiotics, these can be sourced from nature itself. Fungal endophytes are useful resources for active compounds that have a wide range of medical, agricultural and industrial application. Screening the antimicrobial compounds from endophytes is an auspicious way to meet the increasing threat of drug-resistant strains of clinical pathogens. This review article presents some data related to this problem.

Keywords: Antibiotic resistance, Bacterial resistance, Fungal endophytes, Bioactive Compounds, MDR (Multi-Drug Resistance).

INTRODUCTION

Selman Waksman, American microbiologist with his colleagues coined first the term "antibiotics" to define chemical substance which are produced by the microorganisms that have an antagonistic effect on the growth of the other microorganisms[1]. For the human civilization, antibiotics are a blessing as they saved million of people against infections and/ or microbes. Over the years, many different antibiotics have used for therapeutic purposes [2]. Antibiotic-resistant is one of the highest challenges fronting modern medicine [3]. It is reported that antibiotic resistance occurs when the ability of the drug is lost to inhibit the growth of bacteria. Resistant bacteria are the bacteria's that replicate even in the presence of therapeutic levels of the antibiotics[2]. Resistance to antibiotics was known to arise in both pre-antibiotic and antibiotic-era [1]. Generally, antibiotics work by inhibiting the synthesis of cell-wall, nucleic acid or protein of the susceptible species of bacteria or fungi strain. They also act by disrupting the cell membrane or metabolic pathway of pathogens. Either via mutation or by HGT (horizontal gene transfer) from a resistant strain, the bacterium becomes resistant to an antibiotic [4]. They can occur as a natural selection process where all the bacteria were empowered by nature with a low-level degree of resistance. For e.g. a study proved that ampicillin, tetracycline and sulfamethoxazole and trimethoprim (TMP-SMZ) were used commonly for several years, but currently, they no longer role its non-cholera diarrhea disease treatment in Thailand. Another study in Bangladesh reported that the same drugs exhibited an effective treatment [2].

Multidrug-resistant pathogens represent a growing health threat to the public, as the infections caused by the MDR (multi drug resistance) are challenging and the treatment is expensive. And a number of antibacterial and antifungal compounds and fewer antimicrobial agents using a novel mechanism of action are still in clinical development [5]. Initially, the antibiotic resistance was observed in gram-negative bacteria but now with gram-positive and particularly during the last decade. The infections caused by gram-positive bacteria have become a serious issue, especially in the nosocomial setting [6]. Multidrug-resistant *Staphylococcus aureus* is resistant to penicillin-like beta-lactam antibiotics. From minor skin infections to severe life-threatening infections such as meningitis, pneumonia, septicemia, endocarditis, postoperative infections and toxic shock syndrome were caused by MRSA. As the Gram-positive pathogen becomes multidrug resistant, MRSA strains have become a serious issue in healthcare settings worldwide [7,8]. They stated a significant threat to U.S. public health[9]. Including quinolones, Methicillin-resistant *Staphylococcus aureus* is resistant to several other antimicrobial agents and are prevalent in many hospitals. Studies reported from several countries like USA, Europe and Japan about the rise of multiply resistant *S.aureus* with reduced sensitivity to glycopeptides[6]. And still some antibiotics like daptomycin, linezolid, vancomycin, ceftaroline and teicoplanin hold activity against MRSA [9]. Due to the multidrug-resistant bacterial infections, the death rates are high. In the EU, about 25,000 patients died from an infection with the selected multidrug-resistant bacteria and 63,000 patients die from hospital-acquired bacterial infections every year in the US [10]. In US hospitals, the main causative agent's infections are the ESKAPE organism's *K.pneumoniae*, *Enterobacter* species, *Staphylococcus aureus*, *A.baumanii*, *E.faecium* and *P.aeruginosa*. There is a rise in numbers of Penicillin-resistant *Streptococcus pneumoniae*, Vancomycin-resistant *Enterococcus faecium* and Methicillin-resistant *Staphylococcus aureus* in the last two decades [2]. *Enterococcus* faecium and *E.faecalis* cause VRE infections,

when compared to MRSA, VRE has inferior prevalence and epidemiological impact. Quinupristin and linezolid are some of the antibiotics that are used for treating VRE [11]. VRSA was multi-drug resistant against the large variety of presently available antimicrobial agents [12]. To the currently available antibiotics, MRSA and VREF had become resistant[3].

Fungal Endophytes

Fungi are the second largest group with a great biodiversity of an organism that serves as the crucial factor of tropical ecosystems all over the world. Endophytes are bacteria or fungi that reside inside the host plant for a whole or half of its life cycle without causing any apparent diseases [13]. They are abundant microorganisms with greater biodiversity. There are approximately 300.000 species of plants exists on the earth, in which each one of them serves as the host plant for one or more endophytes. Endophytes are remarkable and interesting microorganisms for targeting new products from nature [14]. Endophytes produce a wide range of structural classes of secondary metabolites like steroids, phenols, alkaloids, flavonoids, peptides and quinones and thus they can be also used as antimicrobial agents [15]. In developing countries, 80% of the people depend on drugs obtained from herbs for their initial healthcare. Between 1981 and 2014, more than 51% of drugs were based on products obtained from nature and the rest from synthetic [16]. As a starting material, these naturally occurring biologically active material can be used for agrochemical and pharmaceutical products [17]. About 1 million species from fungal endophytes are present in all plants [18]. Almost all the vascular plant species that has been studied was found to harbor Fungal or bacterial endophytes and are present nearly in all parts of the host plant and some of them are seed-borne [16]. By producing compounds, endophytes directly or indirectly favor the host plant [19].

Diversity of Endophytes

Endophytes are considered to be the most diverse and important resources available for natural products with substantial biological activity [15]. Only a few plants species from 420,000 have been studied completely to the biology of endophytes. The diversity of endophytic fungi is 7% out of a total of 1.5 million fungi on earth [20]. They are distributed extensively and consist of a wide variety of novel secondary metabolites with various biological activities [21]. Plant pathologists, chemists, mycologists, ecologists were attracted towards fungal endophytes, from the first reported isolation from *Lolium temulentum* to the latest from Antarctic moss [22].

Endophytic fungi against multi-drug resistant bacteria

Mada Triandala Siber *et al* reported that fungi MPS 14.1/MT 02, MPS 14.3/MT 04 isolated from marine sponge exhibited significant antibacterial activity with a zone of inhibition of about 17.2 mm^2 and 16.9mm^2 respectively against pathogenic multi-drug resistant *E.coli* [23]. The study reported that three out of six endophytes, i.e., *Cladosporium*, *Colletotrichum* and *Guignardia* isolated from *Aegiceras corniculatum*, showed activity against at least one or more test pathogens (*P. aeruginosa, K. pneumoniae, B. cereus, A. baumanii, E.coli*) [24]. The red pigment isolated from the endophytic Fungi isolated from *Hydnophytum formicarum* have exhibited good antibacterial activity against Multidrug-Resistant *E.coli* with the zone of inhibition of 19.8±1.13 mm was reported by Mada Triandala Sibero *et al* [23]. Another study reported that cytochalasins compound from endophytic fungus *Phomopsis* sp. derived from Garcinia kola was tested for antibacterial activity against test pathogens. In which the antibiotic ampicillin doesn't exhibit any activity against *Shigella flexneri* SDINT, *V. cholerae* NB2 and *V. cholerae* PC2 up to 512 µg/mL Concentrations whereas, these MDR strains were found to be sensitive to cytochalasin metabolites [25]. The compound Ambuic acid and Ambuic acid derivative were isolated from *Pestalotiopsis* sp of *Lichen Clavaroids* sp. exhibited antibacterial activity against *S.aureus* [26]. Colletotric acid isolated from *Colletotrichum gloeosporioides* of the plant *Artemisia mongolica* showed significant antibacterial activity against *S.aureus*, *Bacillus subtilis*, *S.aureus* and *S.lutea* (MIC of 25, 50, and 50µg/mL)[27]. Periconicins A and B from *Periconia* species isolated from *Taxus cuspidata B.subtilis, S.aureus, K.pneumoniae, Salmonella typhimurium* (MICs in the range of 3.12–12.5µg/mL) *and B.subtilis, S.aureus, K.pneumoniae, Salmonella typhimurium* (MICs in the range of 25–50µg/mL) respectively[28]. Alterporriol N and Alterporriol E were isolated from an endophyte *Stemphylium globuliferuman* from *Mentha pulegium* collected from Morocco. Alterporriol N was active against MRSA and *E. faecalis* with MICs of 62.5 and 15.63µg/mL whereas Alterporriol E is active against Enterobacter cloacae and *S.pneumonia* and *E. faecalis* with a minimum inhibitory concentration of 31.25µg/mL [29].

The major cause of resistance is because the antibiotics can be bought without any medical prescription [2]. There is an increase in resistant bacteria to clinical antibiotic use is because of the lack of new antibiotics development [30]. By reducing the strength of natural selection for resistance genes, antibiotic resistance evolution can be slowed down[31]. Need in search of new and novel drugs platforms remains of higher importance in order to fight against these lasting developments [22]. In order to counter the health threats from MDR, a few strategies are being explored. Some of them include enhancement of prescribing practices, developing new artificial and species-specific antibiotics, use of a combination or host-directed therapies and synthetic antibiotics, obtaining new antibiotics by activation of cryptic or silent antibiotic gene clusters of microbes. And also, by identifying novel target sites in bacteria by structural and functional genomics [4]. Endophytes are protected and feed by the plant thereby they produce a biologically active substance, enhancing the plant growth and resistance in the environment [32]. Several structurally diverse metabolites have been isolated from the endophytic fungi that are used for developing

valuable pharmaceutical products [33]. They offer an excess of mysterious advantages to host with massive applications in medicine and agriculture [34]. They provide us with ecofriendly drugs that could be directly harvested from fermentation rather from host trees that lead to deforestation [19]. Fungal endophytes produce several useful compounds which as antimicrobial, anticancer, plant growth hormones, antiviral properties [35].

CONCLUSIONS

Antibiotic-resistant bacterial infections are becoming more predominant and major health crises facing us in today world. They levy a remarkable financial problem on world economies. Secondary metabolites are of major interest because of their applicability as therapeutic agents. It will be important to understand the physiological relevance and ecological significance of secondary metabolites, in order to overcome the continuous emerging of bacterial resistant strains. It is important to decode our growing understanding on mechanisms of antibiotic into new clinical approaches, thereby we can successfully fight against the increasing threat from resistant pathogens.

Acknowledgment: The authors thank Vellore Institute of Technology, Vellore, India for the encouragement, support.

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Source of Support: Nil, **Conflict of Interest:** None.