Journal of Ayurveda and Integrative Medicine xxx (2017) 1–10

Contents lists available at ScienceDirect

Journal of Ayurveda and Integrative Medicine

journal homepage: http://elsevier.com/locate/jaim

Review Article

Development of botanicals to combat antibiotic resistance

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ARTICLE INFO

Article history:
Received 28 November 2016
Received in revised form
9 March 2017
Accepted 23 May 2017
Available online xxx

Keywords:
Antibiotic resistance
Botanicals
Synergism
Immunomodulation
Bioenhancers

ABSTRACT

The discovery of antibiotics in the previous century lead to reduction in mortality and morbidity due to infectious diseases but their inappropriate and irrational use has resulted in emergence of resistant microbial populations. Alteration of target sites, active efflux of drugs and enzymatic degradations are the strategies employed by the pathogenic bacteria to develop intrinsic resistance to antibiotics. This has led to an increased interest in medicinal plants since 25–50% of current pharmaceuticals are plant derived. Crude extracts of medicinal plants could serve as an alternate source of resistance modifying agents owing to the wide variety of secondary metabolites. These metabolites (alkaloids, tannins, polyphenols etc.) could act as potentials for antimicrobials and resistance modifiers. Plant extracts have the ability to bind to protein domains leading to modification or inhibition protein–protein interactions. This enables the herbals to also present themselves as effective modulators of host related cellular processes viz immune response, mitosis, apoptosis and signal transduction. Thus they may exert their activity not only by killing the microorganism but by affecting key events in the pathogenic process, thereby, the bacteria, fungi and viruses may have a reduced ability to develop resistance to botanicals. The article is meant to stimulate research wherein the cidal activity of the extract is not the only parameter considered but other mechanism of action by which plants can combat drug resistant microbes are investigated. The present article emphasizes on mechanisms involved in countering multi drug resistance.

1. Background

Antibiotics, the wonder drugs of the 20th century, play a critical role in treating bacterial infections.

The synthesis of Salvarsan an arsenic based drug for syphilis in 1910 and development of Prontosil, a sulpha drug in 1935 and penicillin purified and produced in early 1940s, set up the paradigms for future drug discovery research. The period from 1950s to 1970s was considered the golden era of discovery of novel antibiotics classes [1]. This resulted in a major reduction in mortality and morbidity, due to infectious diseases leading to a euphoria [2,3]. However, with each passing decade, bacteria that could defy multiple antibiotics started becoming increasingly common, leading to an increase in morbidity, mortality and cost of health care. The increase of drug resistant organisms stemmed from a multitude of factors [4,5]. The improper use of antibiotics in patients contributed to the emergence of drug resistance. Additionally extensive use of antibiotics in the animal industry has also resulted in strong selective pressure for the emergence of antibiotic-resistant bacteria [6–8]. With increasing patient movement and travel throughout the world, transmission of the drug-resistant organisms from one country to another also increased [9,10].

The wide spread antibiotic resistance observed is now posing a serious public health concern, with medical scholars warning of a return to the pre-antibiotic era [11]; be it community or hospital acquired infections due to Vancomycin Intermediate *Staphylococcus aureus* (VISA), Vancomycin Resistant Enterococci (VRE), Methicillin Resistant *S. aureus* (MRSA) or ESBL (extended spectrum β-lactamase) enzyme producing Gram negative bacteria [12]. Thus effective antimicrobials were no longer available which could cure virtually all bacterial infections. This optimism was shaken further by the emergence of resistance to multiple antibiotics amidst enteric pathogens, *Pseudomonas aeruginosa, Streptococcus pneumonia, S. aureus* and *Mycobacterium tuberculosis.* [13–15]. Additionally a high level of drug resistance is reported in *Enterococcus faecium, S. aureus, Klebsiella pneumoniae, Acinetobacter baumannii,*

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Peer review under responsibility of Transdisciplinary University, Bangalore.

http://dx.doi.org/10.1016/j.jaim.2017.05.004

Please cite this article in press as: Gupta PD, Birdi TJ, Development of botanicals to combat antibiotic resistance, J Ayurveda Integr Med (2017), http://dx.doi.org/10.1016/j.jaim.2017.05.004

P. aeruginosa and Enterobacter spp. – together referred by the acronym ESKAPE which cause the majority of infections within the hospital environment [16] The wide range of antimicrobial resistance (AMR) mechanisms used by the ESKAPE pathogens, includes enzymatic inactivation, modification of drug targets, changing cell permeability through porin loss or increase in expression of efflux pumps and mechanical protection provided by biofilm formation. AMR in these pathogens is a major concern to public health systems worldwide and is likely to increase as resistance profiles change.

The damaging effects of AMR are already being observed. AMR infections currently claim at least 50,000 lives annually across Europe and the US. In other areas of the world reliable estimates of the true burden are scarce but it is estimated that the deaths amount to many hundreds of thousands. It is estimated that if there is a continued rise in resistance levels, by 2050 it would lead to 10 million deaths annually [17,18]. Additionally, AMR leads to longer hospital stays, higher rates of hospitalization and rise in the treatment cost [2,19]. Preliminary research which considers only a part of the impact of AMR estimates that by 2050 the economic burden would be 100 trillion USD [17].

2. Mechanism of development of antibiotic resistance

The origin of genes for antibiotic resistance is due to a natural process. The source could be genes encoding resistance in the antibiotic producing bacteria themselves as a mechanism for their own protection or generally due to spontaneous mutations in the bacterial chromosome. The spontaneous mutation frequency for antibiotic resistance is on the order of about 10^{-8}- 10^{-9}. Whilst mutation is a rare event, it does not take long for resistance to develop in a bacterial population owing to the fast growth rate of bacteria and the absolute number of cells attained [20].

Once the development of resistance has occurred, the mutated gene is directly transferred to the bacteria's progeny during replication. In the selective environment of the antibiotic, the wild type are killed and the resistant mutant allowed to flourish, influenced by the rate and pattern of antibiotic use (selective pressure) and influence of the particular resistance on bacterial fitness [21]. Resistance to penicillin in *S. aureus* was observed as early as 1942 after penicillin came into use [11].

As the next generations of antibiotics were developed to overcome the problems of resistance against the available ones, bacteria developed resistance mechanisms to the newer antimicrobial agents [22]. For example, the production of an enzyme penicillinase by *S. aureus* led to penicillin resistance initially. To resist penicillinase, cloxacillin was developed. To contest this antibiotic, the bacteria altered the target site for binding of β-lactam antibiotics i.e. the penicillin binding proteins (PBPs) and this led to the development of MRSA. Presently the bacteria have been reported to be resistant to not only methicillin but also to chloramphenicol, macrolides, aminoglycosides, tetracycline and lincosamides [23].

Multidrug resistance in bacteria occurs by accumulation, of resistance (R) plasmids, transposons, or genes with each coding for resistance to a particular agent, and/or due to the action of multidrug efflux pumps (EP) which can pump out more than one drug type [23]. Development of plasmids for multi drug resistance in pathogenic organisms is a comparatively recent phenomenon which occured after the introduction of antibiotics in the 1940s [22].

A recent database lists more than 20,000 potential resistance genes of nearly 400 different types, predicted mainly from available bacterial genome sequences [24]. Fortunately, the number existing as functional resistance determinants in pathogens is much smaller [11].

The key mechanisms responsible for resistance to antibiotics in bacteria are listed below –

- **Plasmids:** Whilst both chromosomal mutations and/or genetic transfer are responsible for acquisition of resistance, it is the transferable resistance which poses a greater threat as it can achieve much wider dimensions due to rapid dissemination. R plasmids play a vital role in carrying this transferable resistance. A single plasmid can harbor several genes coding for multiple drug resistance. Horizontal gene transfer (HGT) is responsible for the development of antibiotic resistance through the transfer of the so-called mobile genetic elements (MGEs) [25].
- **Inactivation of antibiotic:** Bacteria may produce enzymes that chemically modify or degrade antibiotics and inactivate the drugs [26]. For example, Penicillin resistance in *S. aureus* is because of the production of the enzyme β-lactamase that inactivates the antibiotic by hydrolyzing the β-lactam ring [27].
- **Target site modification:** The molecules that are normally bound by an antibiotic are normally altered or replaced and thus essentially eliminate the drug's targets in bacterial cells. An example of this mechanism is Methicillin resistance in Staphylococci due to the presence of mec A gene which encodes for PBP 2A. It has low affinity for β-lactams, conferring resistance to all β-lactam antibiotics, together with β-lactamase inhibitor combinations (ampicillin/sulbactam), cephalosporins and carbapenems [28].
- **Prevent drug uptake:** The entry ports for the drugs can be eliminated by bacteria by altering permeability [29]. It has been reported that *P. aeruginosa* can develop resistance to imipenem by mutational loss of porin proteins thereby modifying the outer membrane permeability [30].
- **Efflux pumps (EP):**There are 5 super - families of microbial efflux systems viz. NorM, multi-antimicrobial extrusion protein family (MATE), QacA major facilitators (MFS), LmrA, ATP-binding cassettes (ABC), MexAB, QacC small multidrug resistance family (SMR), resistance-nodulation cell division (RND) [31]. These EPs are responsible for the export of antibiotics before they find their intracellular targets. Kaplan [32] has demonstrated that an active drug EP is an effective mechanism of macrolide resistance in *Streptococcus pyogenes*. The resistance is encoded by the mefA gene and is specific for 14- and 15-membered macrolides.
- **Biofilm formation:** Biofilm is formed by a complex aggregation of microbes, wherein the cells are embedded matrix of extracellular polymeric substance (EPS) (self-produced). Production of biofilms through adherence of bacteria to human tissues and medical devices is a major virulence factor associated with increased antibiotic resistance, reduced phagocytosis, and overall persistence of the microorganisms [33]. Additionally, these biofilms being difficult to eradicate, are a source of many intractable infections. The inherent resistance of biofilms to the antibiotics can be attributed to failure of antibiotic to penetrate or slow growth rate of organisms owing to slower metabolism [34].

The combined effects of genetic processes of mutation and selection, fast growth rates and the ability to exchange genes, sum up for the extraordinary rates of adaptation and evolution that can be observed in bacteria. For these reasons bacterial resistance or adaptation to the antibiotic environment seems to occur very quickly in an evolutionary timeframe [21]. Bacteria established mechanisms to resist the next generation antimicrobials that were developed to overcome the difficulties associated with resistance [2]. Tuberculosis which is now considered a global emergency,

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