The war against MDR pathogens: move fungi to the frontline

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The evolution and spread of resistance among pathogenic microbes to different antibiotics currently in use is a global health problem. Attempts are being made to tackle this major health burden by involving policy makers, scientists, healthcare professionals, the general public and industry. Several strategies, including improvement of prescribing practices, use of combination therapies and synthetic antibiotics, and development of species-specific antibiotics have been suggested to retard the evolution of drug resistance. However, most of the new antibiotic molecules which are being prepared to be marketed are only modifications of existing ones, thus lacking novelty in their mechanism of action or target sites. It is reasonable to expect that the introduction of totally new antibiotics would delay the evolution of drug resistance. In this context, the filamentous fungi are a promising source of novel antibiotics. Their diverse biochemical pathways, the range of ecological niches they occupy, and that 8% or less of the 2.2–3.8 million estimated fungal species are known, underscore the now urgent need to screen them for novel antibiotics.

Keywords: Drug resistance, filamentous fungi, novel antibiotics, pathogenic microbes.

THE resistance of pathogenic microbes to almost all the antibiotics currently in use is seen as a global threat to public health[1,2]. Multi-drug resistant (MDR) bacteria and fungi are a major concern worldwide as they may cause severe and fatal infections (www.who.int/drugresistance/en/), and by 2050, could cause 10 million deaths annually[3]. For example, the mathematical model of Sharma *et al.*[4] predicts that the percentage of MDR and extensively drug resistant (XDR) tuberculosis bacteria would rise with time in India, the Philippines, Russia and South Africa. Over the years, many species of human pathogenic bacteria and fungi have developed resistance to antibiotics. These include multidrug-resistant *Acinetobacter*, fluconazole-resistant *Candida*, vancomycin-resistant *Enterococcus* (VRE), fluoroquinolone-resistant *Pseudomonas aeruginosa* (FQRPA), methicillin-resistant *Staphylococcus aureus* (MRSA) and drug-resistant *Mycobacterium tuberculosis*[5]. In February 2017, the World Health Organization (WHO) released a first list of antibiotic resistant 'priority pathogens', including 12 species of bacteria which present the greatest health risk (http://www.who.int/medicines/publications/WHO-PPL-Short_Summary_25Feb-ET_NM_WHO.pdf?ua=1). Fair and Tor[6], and Ventola[2] list the causes for this crisis situation, which include indiscriminate and overuse of antibiotics among humans and in agriculture, and inappropriate prescribing. Furthermore, most antibiotics in current use have been obtained from a minimum set of molecular architectures and their lifetimes have been extended by synthetic modifications[7]. This scenario is compounded by the disinclination of pharmaceutical industries to develop new antibiotics from organisms in nature due to economic and regulatory constraints[3,6,8]. As one of the strategies to control MDR pathogens, here we underscore the need to screen fungi in general, and particularly those from largely unexplored habitats, for novel antibiotics.

Antibiotics in nature

In nature, antibiotics are natural products, primarily of bacteria and fungi, but also from some plants and other organisms; their production is a multi-step process under the control of many genes. The ability to produce and resist antibiotics is considered to have evolved early in the evolution of microbes[2]. In the natural environment, these compounds have roles in giving species an ecological advantage, for example, as antifeedants or natural biocides inhibiting or eliminating other species competing for the same resource. This results in increased fitness of the antibiotic producer in interspecific competitions[9,10]. Experiments using soil bacteria confirm that antibiotics aid the producer by inhibiting the establishment of

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competitors, but they also select for antibiotic resistance among the competitors[11].

Resistance to antibiotics

Antibiotics, whether produced directly by organisms or synthetically, are arguably the most successful therapeutic agents in the treatment of human disease to date, but these are becoming less effective due to the evolution of antibiotic-resistant strains. Antibiotic resistance does develop in nature, but there the selection and transmission of this trait amongst bacteria and fungi has been accelerated by the indiscriminate use of antibiotics. Generally, antibiotics work by inhibiting cell-wall synthesis, protein synthesis or nucleic acid synthesis of the susceptible bacterial or fungal species/strain. They may also act by disrupting a metabolic pathway or cell membrane of the pathogen. A bacterium becomes resistant to an antibiotic either through mutation or by horizontal gene transfer (HGT) from a resistant strain[12,13]. HGT is the major mechanism for the evolution of resistance; the resistance genes are usually located in mobile genetic elements of the bacterium, such as plasmids and transposons which function as vectors transferring resistance genes between individual cells of the same or different species[14]. A bacterium resists antibiotics through enzymatic inactivation of the antibiotic, alteration of the metabolic pathway targeted by an antibiotic, active efflux of the antibiotic[15], or by modification of the antibiotic-binding site[16].

Currently, a few strategies are being explored to counter the major health threat posed by MDR pathogens. These include improvement of prescribing practices[17], use of combination or host-directed therapies[18] and synthetic antibiotics[19], activation of silent or cryptic antibiotic gene clusters of microbes to obtain new antibiotics[20], development of synthetic[6] and species-specific antibiotics[21,22], identification of novel target sites in bacteria by functional and structural genomics[23], and discovery of novel antibiotics through culture-independent approaches[24]. In addition to such laboratory-oriented solutions, incentive programmes have been suggested to push many novel antibiotics from their pre-clinical phase to commercialization phase[25]. According to Venkataraman Ramakrishnan, a Nobel laureate, cooperation between governments at the international level will be the critical step towards solving this crisis[26]. Although antibiotic development through all these initiatives now appears to have a fresh impetus, only 33% of the new antibiotics being studied is likely to become marketable[27]. Most of these are not, however, completely new molecules, but modifications of existing ones. They consequently lack novelty in their modes of action or target sites – attributes essential for effectiveness against resistant pathogens[28].

The ideal scenario is to discover entirely novel antibiotics that can tackle drug-resistant pathogens. Bioprospecting for less-studied and novel organisms can be expected to be particularly promising in this regard. Natural products exhibit enormous chemical diversity with chemicals of unprecedented molecular structures and which are necessarily vested with different bioactivities – all advantages over the combinatorial chemistry approach, thus expanding the base for the search for novel antibiotics. This consideration, and that the synthetic methods have not as yet resulted in any game-changing success[29], heighten the importance and urgency of bioprospecting for novel drugs.

Fungi as a platform for novel antibiotics

A majority of the commercialized antibiotics so far obtained is from soil-dwelling actinobacteria[22]. A consequence of this lack of diversity in the source of antibiotics facilitates the acceleration of the development of drug resistance in the target pathogens. Tackling the antibiotic crisis needs a thorough understanding of the evolution and mode of drug resistance, identification of new genetic and chemical indicators of antibiotic action and resistance, the use of systems biology for antibiotic discovery and finding new chemical scaffolds[30]. Although recent endeavours involving genome analysis could aid in revealing novel scaffolds, it is imperative that we explore untapped natural resources to augment the variety of antibiotics used which, by being hitherto unencountered by the pathogens of concern, may be more effective and so could slow down the evolution of drug resistance[7].

In this context, screening fungi for novel antibiotics appears to be a prime option. Despite the knowledge that fungi produce an extraordinary number of compounds, earlier discoveries of pharmaceutically important compounds from fungi were accidental or due to screening of ubiquitous fungal species[31]. Now, however, the pharmaceutical industry is inclined to study fungi more systematically as they are relatively less explored for novel compounds[32,33] and their biosynthetic pathways are highly diverse[34].

The existence of fungi in a myriad of habitats attests to their superior competitive ability. Survival in different ecological niches necessitates competition with different organisms which again entails elaboration of suitable bioactive compounds. Nearly half of the 33,500 known microbial bioactive metabolites are of fungal origin[24]. The extent of unexplored and unnamed fungal biota is enormous, with only around 120,000 described species compared with an estimated range of 2.2 to 3.8 million actual species on Earth[35]. Such realization and that hitherto actinobacteria have been the principal candidates screened for antibiotic discovery[36] are sufficient justification for the exploration of more fungi for novel antibiotics effective against MDR pathogens[37]. This potential is evidenced by studies on fungi from extreme and less-studied

habitats. For instance, endophytic fungi, asymptomatic endosymbionts of plants have proved to be prolific producers of novel bioactive compounds[38–41]. Similarly, fungi inhabiting marine habitats[36], deep seas[42–45] and mangrove ecosystems[46] elaborate an array of novel compounds, including some active against drug-resistant bacteria. Fungi associated with seaweeds[47] and marine sponges[48,49] are another untapped source of novel compounds and some are active against MRSA[50,51]. Therefore, in addition to the various methods already being devoted to counter the antibiotics crisis, it would be prudent to explore the highly diverse unexplored fungi for novel antibiotics[52].

Apart from endophytes and deep-sea fungi, numerous other ecological niches that have not been explored sufficiently for species diversity have been identified[53] – the basic step *sine qua non* for exploiting their chemical diversity. Although a highly specialized niche may entail lesser interspecific competition, reducing the need for any antibiotic production, fungi from such niches may have novel molecular templates for other products important for their survival. A few fungi from such specialized niches have already been found to produce novel compounds, including antibiotics, reinforcing and substantiating the argument that these unique fungi should be explored assiduously. Examples include salt-tolerant and halophilic fungi[54], endolithic, thermo- and psychrophilic fungi[55,56], fungi associated with insects[57], rumens, tropical peat, corals[58], resins, bryophytes and lichens[59,60], and plant pathogenic fungi[61].

We emphasize that apart from screening fungi from such unusual and less-studied habitats for novel antibiotics and their scaffolds, subjecting them to recently developed methodologies such as the identification of biosynthetic gene clusters[62] using the OSMAC approach[63], triggering the normally unexpressed cryptic genes[64–66] and the synthetic biology approach[67] would be worthwhile.

Altering the fermentation parameters increases the chances of induction of a wider range of compounds from a particular strain of a fungus[68], since not all genes are expressed under one set of culture conditions. For instance, the number of metabolite gene clusters housed by members of the fungal subphylum Pezizomycotina group exceeds the number of compounds produced by them[69]. Challenging a test fungus with strains of drug-resistant microbes may well be expected to induce the production of novel antibiotics by stimulating the expression of otherwise silent genes. Co-culturing also has the potential to reveal novel compounds. For instance, when *Penicillium fuscum* and *Penicillium camembertii*/*clavigerum* are cultured together, several novel macrolides are formed which neither fungus produces when cultured alone[70]; one of these, berkeleylactone A, inhibits MRSA[70]. The need for a focused study of fungal biosynthetic pathways is underscored by the recent work of Nielsen *et al.*[71]; genome mining of just 24 species of *Penicillium* revealed 1317 putative biosynthetic gene clusters involved in compound synthesis. Here, potentially prolific fungal producers of natural products outside the relatively well-studied *Aspergillus* and *Penicillium* species, remain to be identified and subjected to genomic mining and epigenetic induction of silent genes[52]. Another facet is to identify methods to increase the production of bioactive compounds in culture by fungi. Numerous lead compounds of fungal origin remain unutilized since they are not produced in required titres[52], at least by routinely used methodologies.

In the light of the evidence obtained so far, we wish to encourage funding agencies to adopt a more proactive approach to exploring and assessing the potential of fungi in unexplored habitats as a platform for the production of compounds active against drug-resistant bacteria and fungi. We also wish to see pertinent authorities revisiting regulations that currently restrict exploration of the unexplored fungal biota by inhibiting the isolation, culture and movement of fungi[8]. In December 2015, the General Assembly of the International Union of Biological Sciences passed a Resolution drawing the attention of nations to Article 8a of the Nagoya Protocol to the Convention on Biological Diversity, urging them to ensure that in developing national regulations they facilitate the exchange of living materials for non-commercial scientific purposes. This is an issue each nation needs to take on board in the fight against MDR organisms.

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