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Mini Review

Strategies to Manage AMR (Antimicrobial Resistance)

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Abstract

With antimicrobial resistance (AMR) rapidly turning into a major health concern, it becomes imperative to be prepared with an arsenal of antimicrobial agents that would work effectively against such drug resistant forms. The challenge here is to avoid rediscovering the already known compounds (dereplication), to develop compounds or strategies to which further resistance would not emerge rapidly and which are amenable to modifications. Some approaches include combining transitional metal elements with already known antibiotics, developing RNA based antimicrobial agents that show highly target specific action, designing antimicrobial peptides with improved biological activity, combining machine learning with suitable experimental designs that would give highly accurate lead identification and also provide access to new chemical spaces with respect to antibiotics, screening new activities or enhancing existing activities in Actinomycetes, controlling target organism population by growth inhibition.

Keywords: Antibiotic or Antimicrobial Resistance (AMR); RNA; Pathogens

Introduction

Antibiotic or antimicrobial resistance (AMR) is the situation when germs or pathogens no longer get killed by the antibiotics that were designed to kill them. Such microbes continue to grow and reproduce more of such resistant microbes. It leads to longer hospital stays, higher medical costs and increased mortality. The most notorious amongst these resistant forms have been given the moniker "ESKAPE" that stands for *Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Acinetobacter baumanii*. While efforts to discover newer antibiotics continue with little success so far, by 2050, AMR cases are estimated to touch 10 million. It therefore becomes imperative to develop new strategies to overcome AMR. Some of these strategies are as under.

Figure 1: Antibiotic discovery and resistance timeline [1].

Metallo-pharmaceuticals and phytochemicals

Transition-metal compounds in combination with antibiotics have given promising results in dealing with AMR, especially when the resistant organism is a Gram negative form. This approach allows one to use antibiotics that commonly inhibit Gram positive forms, against Gram negative forms also. Silver ions have been found to disrupt multiple bacterial cellular networks and processes, causing the destabilization of the cellular envelope and the production of ROS (Reactive Oxygen Species) in Gram negative bacteria. This multi-targeted antimicrobial mechanism of action can be attributed to silver's thiophilic chemical properties. Ag+ can potentiate the activity of a broad range of antibiotics against Gram-negative bacteria in distinct metabolic states, establishing it as a potent antibiotic adjuvant with beta-lactams, aminoglycosides, quinolones and vancomycin. Even with sublethal Ag+ concentrations, moderate morphological changes in the bacterial membrane and protein aggregates can be visualized through transmission elec-

tron microscopy. Though Ag+ interacts with the microbial cell at multiple sites, resistance has been reported in some cases mainly through over expression of copper-related efflux pumps. However, since antibacterial combination therapies with low doses of Ag+ would induce cell death through action at multiple cellular sites, such approaches could potentially delay the emergence of resistance [2].

Mono-resistance to isoniazid is so far the most common first-line drug resistance in tuberculosis; therefore, it has been a challenge to develop more efficient and effective antimycobacterial drugs that are less toxic to mammalian cells. A combination of compounds (isoniazid/$AgNO_3$) with antimycobacterial activity against an isoniazid-resistant clinical strain of *M. tuberculosis* has been identified. The combination had a significant additive effect (50% decrease of individual MICs) and *in vitro* evaluation of cytotoxicity in mammalian cells showed no toxic effects. The combination of antibiotics with transition metals thus gives a treatment alternative where antibacterial effect is enhanced with the combination of drugs, the concentrations used of both drugs are reduced, and the possibility of adverse effects is considerably reduced, resulting in a better outcome for the patient [3].

Figure 2: (a) Isoniazid, (b) AgNO3 (https://images.app.goo.gl/em7RKhr5h9PCRgbK8. https://images.app.goo.gl/dVexguYjkGZz-3pjb7)

A vast repertoire of untapped plant chemicals and other natural compounds also exists that can be studied for their actions against drug resistant microbes.

RNA based antimicrobial strategies

Several of the non-coding (nc) RNA in bacteria have been under study for development as potential anti-microbial agents. Two such RNA are the synthetic sRNA and the CRISPR guide RNAs. Their strength lies in being highly sequence specific thus minimizing off-target inactivation of commensal microbes. Accordingly, the specific target genes that are to be attacked need to be chosen such that only pathogenic forms are eliminated. Such ideal candidate genes include virulence genes, antimicrobial resistance determinants, mobile genetic elements or genes involved in horizontal transfer. Such RNA-based drugs need to be synthesized in a manner that they can be stably maintained inside the target cell. One approach in this direction could be binding such RNAs with specific proteins. The delivery of such RNA-based drugs into the bacterial cytosol also poses challenges with respect to specificity, selectivity and efficacy. This is where bacteriophages and synthetic, non-viral, nanocarriers have been studied for their use in drug and gene delivery. Further, these RNA-based drugs need to be amenable to rapid modifications in the event of resistance development in the target bacteria. Besides, systems like CRISPR-Cas, that have the potential to target several mRNAs in a coordinate manner, would be ideal [4]. CRISPR-Cas system allows for the sequence-specific targeting and selective removal of individual strains of bacteria. Using genomic sequence information, it has been possible to selectively remove closely related bacterial strains whether in pure or mixed cultures. This could be applied for selectively treating multidrug-resistant infections [5].

Figure 3: CRISPR-Cas 9-guide RNA. (https://en.wikipedia.org/wiki/CRISPR_gene_editing, CC BY-SA 4.0, https://commons.wikimedia.org/w/index.php curid77467670)

Antimicrobial peptides

Antimicrobial peptides (AMPs) or host defence peptides, are biologically active molecules produced by various organisms as an essential component of their innate immune response. They present immense structural diversity, but some common features are: a relatively small size (generally between 12 and 50 amino acid residues), their cationic nature due to multiple Arg or/and Lys residues, and the amphipathic structure due to presence of both hydrophobic and hydrophilic regions. They mainly exert their action through electrostatic interaction with negatively charged molecules on bacterial membrane. Their advantage lies in having broad range of activity, lesser toxicity, and decreased resistance development by their target cells. Yet their disadvantages include the fact that most peptides cannot be administered orally as they are rapidly inactivated by gastrointestinal enzymes, thus requiring subcutaneous or intravenous administration, loss of activity under physiological conditions, large size from point of view of chemical peptide synthesis and hence higher production cost [6].

To date, many methods have been developed to design peptides with improved biological activity, such as: substitutions and deletions of amino acids, determination of structure–activity relationships, e.g., altering secondary structure and stabilization of α-helix [7].

Another approach to improve the peptide antimicrobial properties is reversion of the sequence. That is, a retro analogue has the same configuration of chiral centres but an opposite rank order of amino acid residues. However, this procedure does not always produce enhanced antimicrobial activity. On the contrary, it may cause a decrease in selectivity and increase in toxicity as well [8].

Application of machine learning to vast chemical libraries – the halicin hit

As antimicrobial resistance continues to pose a major threat, the high risk of early discovery of new antibiotics and low return on investment, dereplication (same molecule getting repeatedly discovered) and more failures than leads in engineering next generation versions of existing antibiotics have further aggravated the issue. Machine learning approach involving modern neural molecular representations appears suitable with its ability to : 1.reduce the cost, time and labour for new antibiotic discovery and development and 2.increase the true positive rate of identifying structurally novel compounds with the desired bioactivity, i.e., higher rate of accurate lead identification. These neural network approaches have the ability to map molecules from a chemical library into continuous vectors that are then used to predict the properties of these molecules. These designs give rise to molecular representations that are highly consistent with the desired property, thus improving property prediction accuracy over manually crafted representations [9].

Figure 4: Antimicrobial peptides (https://images.app.goo.gl/ERgDDVvaGLiNjpYA8).

In one such study, a deep neural network model was trained with empirical data analyzing *E. coli* growth inhibition by molecules from a widely available FDA-approved drug library along with a modest natural product library, giving a total of 2,335 molecules. The resulting model was applied to predict antibacterial compounds from the Drug Repurposing Hub. With an accuracy of around 50%, the model eventually identified halicin as a broad-spectrum bactericidal antibiotic. This molecule showed exceptional in vivo efficacy in murine models as well. Further, it displayed growth inhibitory properties against a wide phylogenetic range of pathogens through selective loss of the bacterial transmembrane ΔpH potential. Significantly, halicin shows efficacy against *Clostridioides difficile* and panresistant *Acinetobacter baumannii* infections in murine models. *A. baumannii* has been designated by the World Health Organization as one of the highest priority patho-

gens against which new antibiotics are urgently needed. The low structural similarity of halicin to other known antibiotics indicates that this approach is capable of generalization, thus permitting access to new antibiotic chemistry, i.e., identifying molecules that are structurally distinct from existing antibiotics yet show potent antibiotic activity. More such antibiotics with distinct structures were identified from the ZINC15 database [9].

The success of such discoveries depends heavily on the coupling of these approaches to suitable experimental designs along with critical considerations like 1. training of the model, 2.training data involving active molecules that are structurally different thus having the broadest structural variation possible in the training phase to maximize the probability of successful generalization in new chemical spaces and 3.prediction prioritization based on high prediction score, structural uniqueness relative to clinical antibiotics and low or no toxicity [9].

New secondary metabolites and antimicrobials: revisiting *Streptomycetes*

Figure 5: Mode of action of halicin (https://miro.medium.com/max/220/1*JNyY8N339ekR0VTcSQ0zvA.jpeg).

To overcome dereplication, new screening approaches have been adopted for discovering new bioactive compounds in *Streptomycetes*. These include:

1. Unselective or non-specific methods for screening new activities or enhancing existing activities like classical strategies of changing media components (e.g., Daptomycin), increasing general precursors (metabolic engineering, e.g., FK606, Actinorhodin, Oxytetracycline), inducing stress responses with heat/ethanol/salt/acid shock, nutrient limitations (e.g., Jadomycin B, Validamycin A, Manumycin family, Ectoine, Methylenomycin), obtaining strains that overproduce secondary metabolites by random mutagenesis (e.g., Clavulanic acid, Rapamycin, Actinorhodin, Undecylprodigiosin), ribosomal engineering (the alteration of ribosomal proteins to activate cryptic secondary metabolites in streptomycetes, e.g., Actinorhodin) and the use of small molecules as elicitors of secondary metabolism (e.g., Desferrioxamine B/E, Doxorubicin, Baumycin, Actinorhodin, Undecylprodigiosin, Prodiginin, Streptomycin, Pimaricin, other cryptic compounds), co-culturing (e.g., antifungal activity, Actinorhodin, phenolic polyketides). Differentiation of the antibiotic producer mycelium (MII) as a non-specific method to activate antibiotic production is another approach (e.g., Tienamycin, Rodomycin, Erithromycin, Actinorhodin, Apigenin, Luteolin, microbial transglutaminase) [10].

2. Selective methods or biosynthetic cluster-specific methods that can improve the production of already known molecules like self-resistance engineering (upregulation of self-resistance genes, e.g., Doxorubicin, Daunorubicin, Avermectin, Actinorhodin), regulatory engineering (over expression of activators or elimination of repressors, e.g.,, Actinorhodin, Undecylprodigiosin, Avermectin, Streptomycin, Stambomicin A-D, Chromomycin, Alpomycin), genome mining to search for new biosynthetic pathways, heterologous expression of complex biosynthetic pathways of Streptomyces in *S. lividans, S. albus, S. coelicolor* or *S. avermitilis* as expression hosts (e.g., Streptomycin, Chloramphenicol, Congocidine, CECT 3335 laccase, Mithramycin A, Neothioviridamide, Siamycin-I), combinatorial biosynthesis (novel Paulomycin), chemical modification of existing molecules [10].

Other strategies include : 1.exploring non-cultivated bacteria through next generation sequencing (NGS), isolation chip (iChip), co-cultures, 2.study of unexplored niches to look for new Actinomycetes, 3.study of primary metabolism, vegetative growth and germination in actinobacteria [10].

Control instead of Kill

With constant exposure to antibiotics, the sensitive forms of the target organism get wiped out completely with only the resistant forms remaining alive, reproducing and thereby passing on their resistance to all the subsequent generations. An alternative to this could be the strategy of controlling the target organism population rather than eliminating it completely by use of antibiotics. This way the development of antimicrobial resistance could be delayed or even avoided as both the resistant and sensitive forms continue to survive and reproduce. For this it is important to develop antimicrobial agents that act through growth inhibition of the target organism. One instance of this approach is the strategy of attacking quorum sensing and biofilm formation thus keeping the microbial population under control. Several other such metabolic processes of pathogens could be targeted that would inhibit growth without eliminating the sensitive forms completely.

Conclusion

In an effort to tackle AMR, it now becomes apparent that adherence to classical strategies alone may not suffice. Adopting new approaches in multiple disciplines and domains will be required to augment the existing library of antimicrobial agents.

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