

# “Kryptonite” strategies to weaken the “Super-Bugs” a menace to the world



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### 1. Introduction

With the discovery and clinical use of antibiotics, starting with penicillin in the 1940's, humans have been protected from many bacterial diseases. However, the reckless and rampant use of antibiotics has led to the emergence of antimicrobial resistance (AMR) which has caused treatment failures, increased morbidity, mortality, and skyrocketed treatment costs. The World Health Organization (WHO) has declared AMR as one of the top global health threats in 2019. The Indian National Health Policy of 2017 acknowledged the threat posed by antimicrobial resistance (AMR) by recognizing the need for rapid standardization of guidelines regarding the use of antibiotics, to limit the use of antibiotics as growth promoters in animal livestock and to recommend pharmacovigilance of prescription audits with respect to antibiotic usage in the community and in healthcare facilities.

Hitherto susceptible bacteria can develop resistance to antibiotics- “acquired resistance”, by casual mutation or by procuring external genetic material through horizontal gene exchange from other bacteria via plasmids or transposons (1). There are 5 mechanisms of antibiotic resistance (1); (i) inactivation of the antibiotic by enzymes (ii) antibiotic extrusion from the bacterial cell by efflux pumps (iii) decreased uptake of the antibiotic by changes in bacterial membrane permeability (iv) modification of the antibiotic targeting moiety, and (v) development of alternative metabolic pathways.

Besides bringing about awareness to decelerate the progression of AMR by optimizing the use of antibiotics and maintaining hygienic environments, scientists in India and from around the globe are working on strategies to combat the “super bugs” that have emerged which are non-susceptible or resistant to available generations of antibiotics (Figure 1). The approaches include 1) Development of newer antibiotics 2) Development of superior antibiotic delivery systems 3) Inhibition of biofilm formation 4) Co-administration of antibiotic adjuvants 5) Bacteriophage therapy 6) Maintenance of a microbiome.

### 2. Strategies to combat the development of antimicrobial resistance (AMR)

#### 2.1. Strategy 1: Development of newer antibiotics

In 2016, the WHO member states requested the organization to create a priority list of antibiotic resistant bacteria to direct research and development of new and effective drugs. The list is tabulated in the article by Tacconelli and co-authors (2). The WHO centre in India,

collaborated with the Department of Biotechnology to develop a list of drug resistant microbial strains of national relevance to propel research in Indian research centres and pharmaceutical companies to come up with newer drugs to combat the problem of AMR in the country (Table 1). Mycobacteria (including *Mycobacterium tuberculosis*) drug resistant pathogens are not included in the list as these strains are well recognized as global and national threat for which new treatments are required urgently.

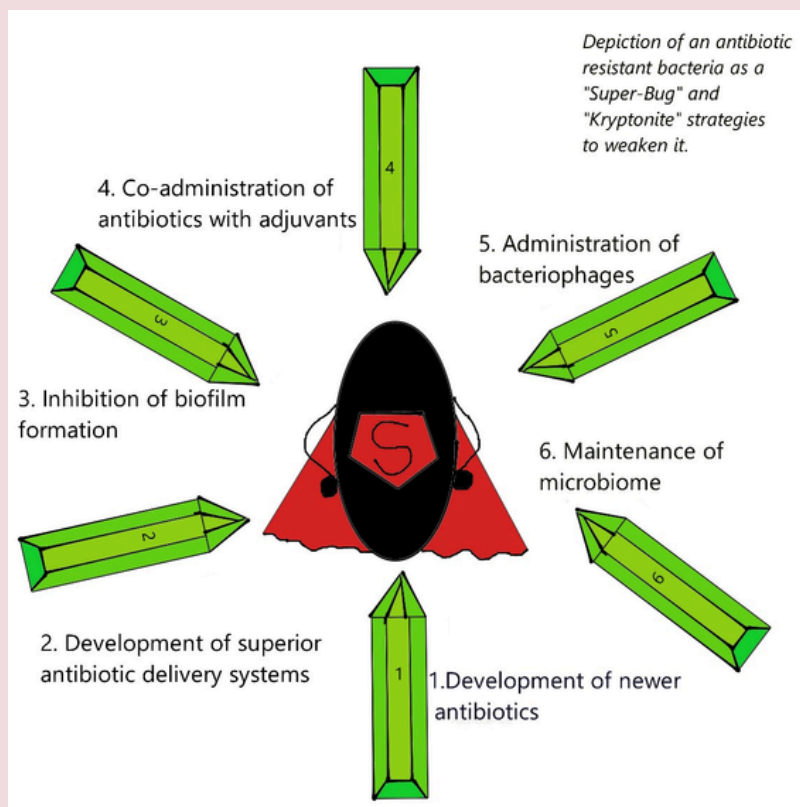


Figure 1: Strategies to combat the development of antimicrobial resistance (AMR)

To tackle the avalanching problem of AMR, new antibiotics or their combinations thereof for treatment of microbial infections have been explored and approved for human use by US FDA (United States Food and Drug Administration) since 2016 (Table 2). Many of these belong to existing classes of antibiotics based on their structural chemistry and mechanism of action. With computer aided design, rational drug design approaches and the advent of artificial intelligence to aid the drug development process, discovery of new antibiotics will be accelerated. We should be able to match the evolution of disease-causing microbial cells with the revolution of newer medicines and mechanisms to stump their growth and pathogenicity.

## 2.2. Strategy 2: Development of superior antibiotic delivery systems

Nanoparticles (Nps) are of small size (1~100 nm) with high surface area-to-volume ratio which enables them to interact with bacteria and cross both bacterial and mammalian cell membranes. “Nanobiotics” which is used to describe nano-systems loaded with antibiotics may be considered superior antibiotic delivery systems (5). Nanobiotics permit higher drug concentrations at infection sites and boost antibiotic association with the bacteria (6). Nano biotics may evade the antimicrobial resistance mechanisms by facilitating the passage through the microbial membrane by altering the shape, size of membrane, interfere with bacterial biological pathways, interfere with bacterial enzymes, deactivate proteins, cause oxidative

stress within bacterial cell, cause an electrolyte imbalance and interfere with the development of genetic variants (6). Nanobiotics may be created by adsorbing, covalently coupling, encapsulating or entrapping antibiotics in nanocarriers (liposomes, dendrimers, cyclodextrins, polymeric, metallic nanoparticles) to improve their pharmacokinetic, pharmacodynamic properties (7).

Antibiotics conjugated with metallic nanoparticles with inherent anti-bacterial activity like silver and gold nanoparticles show improved potency, for example, penicillin conjugated to silver nanoparticles. When penicillin G and erythromycin were combined with silver nanoparticles, an increased antibiotic activity against *E. coli* was observed in-vitro with respect to the diameter of inhibition zones (12mm (with antibiotic-silver Nps), as against 8mm with only the antibiotic) (7).

Antibiotic potency of several antibiotics (macrolides, rifamycins, quinolones, beta-lactams, cephalosporins and tetracyclines) has shown a marked improvement when incorporated into nano-systems of beta-cyclodextrins and their derivatives (8). Minimum inhibitory concentration (MIC) of ampicillin, amoxicillin was decreased by 4-fold and cefadroxil MIC by 16-fold against *Staphylococcus* spp., *Klebsiella* spp., *Escherichia.coli*, *Pseudomonas aeruginosa*, *Enterobacter* spp., *Citrobacter* spp., when the antibiotics were formulated with beta-cyclodextrin carriers (9). The beta-cyclodextrin carriers enhanced the stability and solubility of the antibiotics and probably improved antibiotic permeation through bacterial membranes (8). Liposomes are attractive nano delivery systems and can incorporate both hydrophobic and hydrophilic drugs. Moreover, they are mainly composed of relatively biocompatible and biodegradable systems and do not generally pose problems of toxicity or activation of the immune system (5). The US FDA has approved some liposomal antibiotic formulations and some of these are undergoing clinical trials. Arikayce<sup>®</sup>, Arikace<sup>™</sup> and ALIS are amikacin liposomal formulations that have been approved by the FDA against *Mycobacterium avium* complex (MAC) lung disease, *Pseudomonas aeruginosa* infection (cystic fibrosis patients) and non-tuberculosis mycobacterial lung infections respectively (5). Lipoquin (NCT00889967) and Pulmaquin (NCT02104245) are ciprofloxacin liposomal formulations currently under clinical trials.

### **2.3. Strategy 3: Inhibition of biofilm formation**

Biofilm formation is one of the main reasons of bacterial resistance. A biofilm is an ecosystem of heterogenous sessile bacteria wrapped in an extracellular polymeric substance (EPS) which supports its existence and flourishing. The sessile bacteria can survive and reproduce in harsh environments with the EPS forming a physicochemical barrier guarding the bacterial colonization against anti-microbial exposure and preventing diffusion of antibiotic to therapeutic concentrations within the film, thus spurring resistance to the antibiotic. Bacteria in biofilms are 1000 times less sensitive to antibiotics than bacteria in a planktonic state (10). Formation of antibiotic tolerant biofilms within the host threaten the efficacy of therapy and cause recurrent chronic infections. New chemotherapeutic agents that can prevent or inhibit biofilm formation, maturation and break up mature biofilms are required, so that the conventional drugs can gain access to bacteria and destroy them. The most common biofilm-forming bacteria include *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*,

Staphylococcus aureus, Klebsiella pneumoniae, Enterococcus faecalis, Streptococcus viridans, Escherichia coli, and Proteus mirabilis (11). For inhibiting biofilm formation, the bacterial surface adhesions must be not permitted, the quorum-sensing systems should be disrupted, second nucleotide messenger signalling should be interfered with, biofilm maturation must be inhibited, and mature biofilms should be dispersed (11, 12). A thorough knowledge understanding of biofilm formation and maturation is now leading the way for discovery of molecules that could disrupt the process (12). Preliminary research has led to discovery of compounds such as N-acylhomoserine lactone analogues, patriniae, quercetin, parthenolide, phloretin, hordenine, cinnamaldehyde, ginkgolic acids (GAs) and plant extracts (Hymoenocallis littoralis, Rhodomyrtus tomentosa, Piper betle, Bergenia crassifolia, Zingiber officinale) that have the potential to inhibit biofilm formation (13). Antibiofilm agents are EPS degrading enzymes, anti-microbial peptides, anti-quorum sensing molecules, compounds targeting cellular components and secondary metabolites (14).

Table 1: Indian priority list of antibiotic resistant bacteria (3) (Indian Priority Pathogen List, 2021)

Priority	Bacteria	Antibiotic
Critical	Enterobacteriaceae ( <i>Klebsiella pneumoniae</i> and <i>Escherichia coli</i> )	Carbapenem – R Tigecycline – R Colistin – R
	Non-fermenting bacteria ( <i>Acinetobacter baumannii</i> and <i>Pseudomonas aeruginosa</i> )	Carbapenem – R Colistin – R
High	<i>Staphylococcus aureus</i>	MRSA, hVISA Daptomycin – NS Linezolid – R
	<i>Enterococcus species</i>	Vancomycin – R Linezolid – R Daptomycin – NS
	<i>Salmonella species</i> (Typhoidal and Non-typhoidal)	Azithromycin – NS Third generation cephalosporins – NS Carbapenem – NS
Medium	<i>Streptococcus pneumoniae</i>	Cephalosporin – R Fluoroquinolones – R Linezolid – R
	<i>Staphylococcus, coagulase-negative</i>	Vancomycin – R Linezolid – R
	<i>Shigella species</i>	Third generation cephalosporins – R Azithromycin – R
	<i>Haemophilus influenzae</i>	Third generation cephalosporin – NS Carbapenem – NS
	<i>Neisseria meningitidis</i>	Fluoroquinolones – NS Third generation cephalosporins – NS
R - Resistant, NS - Nonsusceptible, MRSA - methicillin resistant <i>Staph. aureus</i> , hVISA - heterogenous vancomycin intermediate <i>Staph. Aureus</i>		

Table 2: New Antibiotics introduced in the market since 2016 (4).

Class of Antibiotics	Basic Chemical Structure	New Antibiotics or their combinations (FDA approved year) since 2016
<b>DNA topoisomerase IV inhibitors (Antibacterial)</b>	Quinolones	Nemonoxacin (2016), Ozenoxacin (2017), Delafloxacin meglumine (2017), Levonadifloxacin arginine salt (2019), Alalevonadifloxacin mesylate (2019), Lascufloxacin hydrochloride (2019).
<b>Protein synthesis inhibitors acting on ribosomal subunits (Antibacterial)</b>	Tetracyclines	Sarecycline hydrochloride (2019), Omadacycline (2018), Eravacycline (2018)
	Aminoglycosides	Plazomicin (2018)
	Oxazolidinones	Contezolid (2021)
	Pleuromutilins	Lefamulin (2019)
<b>Interference with bacterial cell wall synthesis</b>	Cephalosporins	Cifiderocol (2019).
	Carbapenems	Meropenem/vaborbactam (2017), Imipenem/cilastatin/relebactam (2019)
<b>Interference with fungal cell membrane synthesis</b>	Triterpenoids	lbrexafungerp (2021)
<b>Anti-tuberculosis Drugs- Cell synthesis Inhibitor</b>	Nitroimidazole	Pretomanid (2019)

#### 2.4. Strategy 4: Co-administration of adjuvants

Antibiotic adjuvants are molecules which possess weak or absent antimicrobial activity. These molecules can enhance the activity of antibiotics and minimize or block resistance development in the bacteria. These can also block intrinsic resistance expanding the activity of the combination (antibiotic plus adjuvant) to a wider range of micro-organisms. Antibiotic adjuvants fall under three categories: beta -lactamase inhibitors, efflux pump inhibitors and outer membrane permeabilizers. Beta-lactamase inhibitors include clavulanic acid, sulfobactam, tazobactam, diazabicyclooctane (DBO) among others. Efflux pump inhibitors comprise of phenothiazine, arylpiperazine, quinoline, thioridazine derivatives. Polimyxin B, colistin, aminoglycosides, polycationic/cationic antimicrobial peptides, glycine basic peptide (GBP), caragenins, menadione are examples of membrane permeabilizer antibiotic adjuvants (1).

#### 2.5. Strategy 5: Bacteriophage therapy

Phage therapy advocates the use of bacteriophage to treat bacterial infection (15). Antibiotics are chemical compounds while bacteriophages are complex biological entities that are hosted in bacteria. In view of an upsurge in antimicrobial resistant infections, bacteriophages are being looked upon as suitable alternatives to treat antibiotic-unresponsive disease. Phages are categorized as lytic (causing destruction of bacterial cells) or lysogenic (inserting their genetic material into the bacterial genome) depending on their development cycle. Lytic phages are preferred in a therapeutic context against bacterial infections (16).

Phages are specific in their invasion of bacteria (strain specific), most phages will infect only those bacteria which carry the complementary receptor, while a few phage members can infect a plethora of bacteria (17). The exclusivity of phages for certain strains of bacteria before they

are used therapeutically. Phage libraries are screened for the suitable virus against the given bacterial strain in-vitro before therapy is initiated.

Phage therapy may be used alone or in combination with probiotics, antibiotics or synbiotics. Phage-antibiotic synergy (PAS) refers to an interaction between the two components which potentiates the activity of both and is more efficacious. The phenomenon of PAS may be attributed to; cell elongation or filamentation by antibiotics, increased plaque size mediated by antibiotics, decreased development of phage/antibiotic resistant mutant, increased antibiotic susceptibility, lowered MIC of antibiotics, depolymerization of bacterial polysaccharides to increase antibiotic diffusion and penetration (18).

There are two situations in which phage therapy can be employed, first is compassionate use when the bacterial infection is life-threatening and cannot be controlled by approved medication and methods. The second situation is in a clinical trial setting to determine the safety, efficacy, dosage, and other clinical parameters of the treatment. While the use of bacteriophages in cases of compassionate use have caused relief and the patient has been cured of the infection (19), clinical trials conducted have not yet been able to prove significant advantages of the phage therapy. Currently few clinical trials have been conducted for phage therapy; PhagoBurn (NCT02116010)-used a cocktail of phages against *Escherichia coli* and *Pseudomonas aeruginosa* and the drug silver sulfadiazine and oral phage therapy against diarrhoea (a T4 cocktail phage employed) (NCT00937274). Other clinical trials on therapeutic bacteriophages against *Staphylococcus aureus*, conducted or underway are NCT00663091, NCT04787250, NCT0432345, NCT02664740 (20).

## **2.6. Strategy 6: Maintenance of microbiome**

Microbiome or microbiota refers to the microbial communities of the gastrointestinal (GI) tract, “gut microbiota” and of the mouth “oral microbiota”. The GI tract contains a vast majority of bacteria, archae, viruses, phages, yeast and fungi (21). Gut microbiota is essential for the proper development of the intestinal tract and maturation of the immune and nervous system (22). The normal gut microbiota does not permit the colonization and proliferation of pathogenic bacteria, but if there is an irrational use of antibiotics, gut microbiota loses its diversity and the patient is susceptible to infections of GIT, diarrhoea and colitis (23). The microbiota exerts anti-microbial effects on pathogenic bacteria by competing for nutritional/metabolic resources, producing a wide range of inhibitory compounds (anti-microbial peptides and bile acids), and cause lysis of specific invading bacteria via phages and viruses that are part of the gut microbiome. Further, the microbiota maintains a mucosal barrier, primes the innate immune defence system and, produces cytokines that perturb the colonization of the invader (24).

Modulation of gut microbiota can be achieved through diet, probiotics (naturally occurring live micro-organisms eg. Yakult, SYMPROVE™), prebiotics (substrates that are used by host micro-organisms), synbiotics (a combination of prebiotic and probiotic). Gut microbiota may also be replaced or replenished by a procedure called faecal microbiota transplantation (FMT).

In this procedure, stool from screened healthy donors is administered to recipients for improving their health. FMT is recommended for patients with recurrent *Clostridium difficile* infections. FMT re-establishes the health microbiota and hinders the proliferation of *Clostridium difficile*. There are several clinical trials that are investigating FMT for eradication of GIT of other resistant organisms (NCT05632315, NCT03802461, NCT04188743, NCT04181112, NCT04746222, NCT04759001, NCT04431934, NCT04760665, NCT04146337, EUCTR2019-001618-41, NCT03063437, NCT03061097, EUCTR2019-004402-10-FR) (24).

### 3. Conclusion

Although there are several counter-offensive measures to combat AMR organisms, and an ever-growing need for antibiotics, health care practitioners and patients should be disciplined when prescribing or administering the medicine respectively. If bacteria are exposed to antibiotics below the minimum bactericidal concentration, they have the propensity to mutate and turn resistant. Resistance could be acquired from plasmids from other neighbouring microorganisms or by mutation within the bacterial chromosome itself. Medical practitioners should not prescribe the newest generation of antibiotics, when the infection can be curtailed by an older antibiotic and the patients should complete the entire course of the antibiotic and not stop taking the treatment as the symptoms of infection subside.

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# Fun & frolic – Crossword

Solution from page 9

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**Across**

1. First drug against which resistance was identified (**tetracycline**)
5. Hospital-Acquired Infections (**nosocomial**)
6. Drug cleaved by beta lactamase (**penicillin**)
7. Antimetabolite (**dapsone**)
8. ....Resistant K. pneumoniae is the pathogen on the CDC threat list (**carbapenem**)
9. Antibiotic against cell wall synthesis (**bacitracin**)
10. Drug affected by mutation in RNA polymerase (**rifampin**)

**Down**

2. E of ESKAPE (**enterococcusfaecium**)
3. Responsible for antimicrobial resistance (**effluxpump**)
4. Recent bacterial secondary metabolite drug against gram negative superbugs (**darobactin**)