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Antibiotic adjuvants as a novel strategy to tackle antibiotic resistance

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Abstract
Today antimicrobial resistance is considered as one of the most important threat to global health. So it is very crucial to identify measures to overcome the different mechanisms of antibiotic resistance. Among the major approaches to tackle Antibiotic resistance, antibiotic adjuvant strategies play an important role. The antibiotic adjuvant combinations that attained clinical success include β lactamase inhibitors, efflux pump inhibitors, outer membrane permiabilisers, virulence inhibitors and nanoparticles. Preclinical and clinical trials on these different approaches are going on.

Keywords: Antimicrobial resistance, antibiotic adjuvant strategies, β lactamase inhibitors, efflux pump inhibitors, outer membrane permiabilisers, virulence inhibitors and nanoparticles

1. Introduction
Discovery of antibiotics has been considered as the one of the most relevant contributions of the 20th century. With the use of antimicrobials, a number of infectious diseases were controlled and even eradicated. But the overuse or misuse of these antibiotics in human beings, has led to the emergence of antibiotic resistance [1]. The problem of antibiotic resistance is equally important in animals as antibiotics are used extensively in them as therapeutic, prophylactic or as a growth promoter [2]. Widespread usage of antibiotics resulted in a selection pressure for bacteria to develop mutations or acquire resistance genes [3]. Today antimicrobial resistance is considered as one of the most important threat to global health [4].
Resistance can be divided in to two groups, intrinsic resistance or acquired resistance. While intrinsic resistance is due to the absence of targets to the antibiotics, acquired resistance is due to casual mutations or through acquisition of external genetic material. Horizontal gene exchange occurs through plasmids or transposons. Bacteria develop resistance to antibiotics through different mechanisms 1. Altering drug uptake by changes in the outer membrane permeability 2. Expression of efflux pumps for pumping out antibiotics 3. Modification of the drug target 4. Enzymatic inactivation of antibiotics to make them inactive 5. Through virulence factors [5].

2. Current scenario
World Health Organizaion (WHO) recently published an extensive list of antimicrobial resistant bacteria and the resistance level is very high in the case of almost all the clinically relevant bacteria. These bacteria are resistant to almost all antibiotics in the current pipeline. So it is very crucial to identify measures to overcome the different mechanisms of antibiotic resistance. The global consumption of antimicrobials in livestock was estimated to be 63,151 units in 2010. India accounts for 3% of the global consumption and is the fourth highest in the world. At 12.9×10^9 units of antibiotics consumed in 2010, India was the largest consumer of antibiotics for human health [6]. In January 2010, Food Safety and Standards Authority of India (FSSAI) limited the use of antibiotics in livestock rearing. An indicator of the rising tide of AMR in India is the rapidly increasing proportion of isolates of *Staphylococcus aureus*that are resistant to methicillin. In 2008, about 29% of isolates were of methicillin - resistant *Staphylococcus aureus* (MRSA), and by 2014, this had risen to 47% [7].
Presence of antimicrobial residues in food animal products were reported from different parts of India [8]. Similaror related bacterial strains of animal origin were reported in human population [9]. Vancomycin resistant *S. Aureus*isolated from milk samples[10] Gram negative organisms were isolated from milk samples among cattle suffering from mastitis, of which 48% were ESBL producers [11].

Presence of large number of resistant bacteria and corresponding genes in animal food products were reported [12].

3. Surveillance and monitoring of antibiotic resistance

In May 2015 World Health Assembly adopted a global action plan on antimicrobial resistance (GAP-AMR), which outlines five objectives [13]: `

- to improve awareness and understanding of antimicrobial resistance through effective communication, education and training; `
- to strengthen the knowledge and evidence base through surveillance and research; `
- to reduce the incidence of infection through effective sanitation, hygiene and infection prevention measures; `
- to optimize the use of antimicrobial medicines in human and animal health; `
- to develop the economic case for sustainable investment that takes account of the needs of all countries and to increase investment in new medicines, diagnostic tools, vaccines and other interventions.

3.1 Elements and execution

WHO has initiated many programmes with the above mentioned objectives to fight antimicrobial resistance

3.1.1 World Antimicrobial Awareness Week (WAAW): Held annually since 2015, WAAW is a global campaign that aims to increase awareness of antimicrobial resistance worldwide and to encourage best practices among the general public, health workers and policy makers to avoid the further emergence and spread of drug-resistant infections. WAAW takes place every year from 18 to 24 November. "Antimicrobials: Handle with Care" is the slogan in 2020.

3.1.2 Global Antimicrobial Resistance and Use Surveillance System-(GLASS): Coordinates collection, analysis and sharing of data related to antimicrobial resistance at a global level

3.1.3 Global Antibiotic Research and Development Partnership-GARDP: Encourages research and development through public-private partnerships.

3.1.3 Interagency Coordination Group on Antimicrobial Resistance (IACG): to improve coordination between international organizations and to ensure effective global action against this threat to health security.

Along with the GAP-AMR, National Action Plan to contain AMR (NAP-AMR) has been initiated in India in May,2017.

3.1.4 The National Programme on the Containment of Antimicrobial Resistance: launched under the aegis of the National Centre for Disease Control (NCDC) under the Twelfth Five-Year Plan (2012–2017). The objectives of this programme were to establish a laboratory based AMR surveillance system of 30 network laboratories, generate quality data on AMR for pathogens of public health importance, strengthen infection control guidelines and practices, and promote rational use of antibiotics; and generate awareness about the use of antibiotics in both health care providers and in the community

The Indian Council of Medical Research (ICMR) has established a national network on surveillance of AMR in laboratories based at academic centres, targeting medically important index microbes which have been identified by WHO.

3.1.5 The Antimicrobial Resistance Surveillance Research Network (AMRSN): established by the ICMR has six reference labs for six pathogenic groups that are located in four tertiary care medical institutions. ICMR has also developed a real time online AMR data entry system for its network and will have AMR data analysis capacity specific for bacterial species in relation to point of origin of pathogens as well as various patient demographic parameters. The AMRSN, although currently limited to human health, plans to scale up on a national scale and expand its ambit to include samples from a wider spectrum of sources, including animal, environmental and food samples, to reflect the One Health Approach.

3.1.6 Antimicrobial Stewardship, Prevention of Infection and Control (ASPIC) in 2012: ICMR launched the programme on Antimicrobial Stewardship, Prevention of Infection and Control (ASPIC) in 2012 through collaboration between the office of the National Chair of Clinical Pharmacology, ICMR and the Christian Medical College, Vellore

Along with NAP-AMR, Kerala became the first state to launch action plan on antimicrobial resistance - Kerala Antimicrobial Resistance Strategic Action Plan (KARSAP) in 2019 [14].

3.2 Antibiotic adjutants –definition

Among the major approaches to tackle Antibiotic resistance, antibiotic adjuvant strategies play an important role.Combination therapy of antibiotics and adjuvants has been identified as the recent advancement in the fight against antibiotic resistance, which helps in suppressing antibiotic resistance and enhance antibiotic activity. Antibiotic adjuvants are compounds with weak/no antibacterial activity and they aid in suppressing resistance and enhance antibiotic activity.

3.3 Antibiotic adjutants – review of the current system

The antibiotic adjuvant combinations that attained clinical success include β lactamase inhibitors, efflux pump inhibitors, outer membrane permiabilisers and virulence inhibitors [15].

3.3.1 β lactamase inhibitors

Beta lactamases are enzymes produced by many relevant bacteria which can inactivate β lactam antibiotics through hydrolysis. Inhibitors can be combined with a specific β lactam antibiotic to inhibit β lactamase enzymes.β lactam - β lactamase inhibitor combinations were identified that inactivate β lactam anibioicseg. Clavulanic acid with amoxicillin, Sulbactam withampicicillin and cefoperazone, Tazobactam with cefoperazone.Non β lactam- β lactamase inhibitor combinations includeAvibactamwith ceftazidime/ ceftaroline, Nacubactam/relebactam with imepenam, boronic acids.Synthetic Non β lactam inhibitor VNRX 5133 with Cefepime showed antibacterial activity in carbapenem resistant enterobacteriaceae [16].

3.3.2 Efflux pump inhibitors

This type of resistance mechanisms involve the antibiotics that exert their antibacterial action inside the bacterial cell eg.

fluroquinolones, tetracyclines, macrolides. These are bacterial transport proteins involved in extrusion of substrates from the cellular interior to the external enviornment. The efflux pumps are classified based on sequence similarity, substrate specificity and energy source.Primary efflux pumps draw energy from ATP hydrolysis eg. ATP binding cassettes. Secondary efflux pumps draw energy from chemical gradient eg. Small multidrug resistance family (MRS),Multidrug and toxin extrusion family (MATE), Major facilitator superfamily (MFS), Resistance nodulation cell division (RND).A single efflux pump can extrude a wide range of antibiotics and so their inhibition can improve bacterial susceptibility to a number of antibiotics [17].

There exists different possibilities for the action of the efflux pumps [18]. It can be through the Inhibition of the energy sources required for the activity of the efflux pumps; the membrane poenial and the generation of ATP. Another mechanism is by developing compounds able to compete with the antibiotics for their extrusion eg.a. Phenyl alanine – arginine-β naphthylamide (PAβN) inhibit RND efflux pumps, but many reports of toxicity of the compounds exist [19]. Other molecules in this group includePyridopyrimidines and arylpiperazines and their therapeutic efficacy studies have been reported [20]. Another method of efflux pump inhibition is by modification of the antibiotics to reduce its affinity for the efflux pumps eg. New compounds of the glycylcycline and ketolideclasses differ from their progenitors in showing lower affinity to specific efflux pumps [21].

Efflux pump inhibitors with no definite mode of action are classified based on their source. Plant derived EPIs include a wide variety of molecules that synergestically enhance antibiotic efficacy. Major subclasses are as follows.

a. Plant alkaloids – Reserpine from *Rawolfiaserpentina*is a promising EPI that potentiated the action of tetracycline in *B. subtilis* [22] and norfloxacin in *S.Aureus* [23]. The efflux pump inhibitory action of piperine from *Piper nigram*and its derivatives has been reported against *S. aureus* and *Mycobacteria* spp [24, 25].
b. Flavonoids- Baicalein a weak antimicrobial flavones isolated from thyme leaves (*Thymus vulgaris*) improved the susceptibility of clinical MRSA strains towards ciprofloxacin and β lactam antibiotics [26].
c. Polyphenols- Catechingallates such as epicatechingallate and epigallocatechingallate (green tea leaves) are weak inhibitors of NorA efflux pump [27].
d. Phenolic diterpenessuch as carnosol from the herb rosemary (*Rosemarinus officinalis*) has shown efflux pump inhibition activity for tetracycline and erythromycin against macrolide resistant strain of *S. aureus* [28].

b. Synthetic origin: Synthetic small molecule EPIs are further classified as follows.

1. Peptidomimetic compounds: The dipeptide amide compound PAβN was one of the first EPIs discovered. PAβN has been reported to potentiate the activity of antibiotics fluoroquinolones, macrolides and chloramphenicol [29].
2. Quinoline derivatives - Quinoline derivatives such as pyridoquinolones can restore the activity of norfloxacin in *E. Aerogenes* [30].
3. Aryl piperidines /pipezraines– Phenylpiperidines inhibited the action of *S. aureus* MDR efflux pumps [31].

A small number of EPIs were produced from microbes eg. LA 371 α and LA 371 δ produced from fermentation extract of *Streptomyces* spp [32].

3.3.3 Outer membrane permiabilizers

As the outer membranes in Gram- bacteriae mainly composed of polyanionic lipopolysacharides and porins, which limits antibiotics in to the cell, some antibacterials have reduced efficacy in treatment. Permiabilisers interact with -vely charged outer membrane and disrupts the barrier. Permiabilizers are cationic molecules which Interact with polyanionic lipopolysacharides and destabilises the cell wall eg. polymyxin, Colistin, aminoglycosides [33]. Chemosensitizers which disrupt membrane protein activities have also been proved as outermembrane permiabilisers.eg. Detergents, surfactants.It has been reported that a glycine basic peptide (GBP), a cationic polypeptide. works by disrupting the membrane barrier and the *E. coli* ion-channel and improved the sensitivity of *E. coli* to erythromycin and rifampicin. [34]. Another study revealed the effect of menadione on the membrane permeability of MDR strains of *S. aureus, P. aeruginosa, and E. coli.* [35]. Endogenous antimicrobial peptides (AMPs), which are factors secreted by host cells and organs (e.g., neutrophils, exocrine glands, etc.). destabilize the outer cell membrane of prokaryotes by the formation of an amphipathic αhelix or short β sheet structures [36]. But their therapeutic use remains uncertain due to the high cost of their production and also, the proteases secreted by bacteria have been shown to neutralize AMP's activity. Caragenins, a new class of adjuvants, which are cationic steroidal antibiotics, are resistant to the action of proteases. Positively charged caragenins, gets attracted to the negatively charged membranes leading to cell death through disruption of the membrane It was demonstrated that by combining CSA-13 with antibiotics, synergy was achieved with colistin (55%) and tobramycin (35%) [37].

3.3.4 Anti virulence factors

Virulence factors are expressed in bacteria only during infections. They are non essential for the basal growth of the bacteria but they are essential for disease causation by the bacteria and hence targeting these antivirulence factors can effectively inhibit the ability of the bacteria to cause infections [38, 39].

a. Targeting biofilms

Biofilms are the predominant life-mode of most bacterial species which are densely packed microcolonies concealed in a protective matrix of biopolymers [40, 41]. Bacterial species employ so-called c-di-GMP signalling to produce an extracellular matrix and form biofilm, or assume a planktonic lifestyle [42, 43]. A reduction in the c-di-GMP level down regulates the production of biofilm matrix components and causes dispersal of biofilm [44]. Biofilm inhibitors include a. compounds that modulate the function of pili and curli in *Escherichia coli*b. Compounds that are modulators of c-di-GMP Signalling and c. compounds that target Quorum sensing. Bacterial attachment is the first step in biofilm formation and failure to attach to surfaces results in eradication of the infection. Bacterial pili and fimbriae systems are involved in surface attachment and facilitates colonization of the underlying tissue [45]. Pili are often assembled via a chaperone usher pathway [46]. Construction of

pili occurs by the attachment of the functional subunits from top to bottom [47]. Pilicides are compounds that interfere with pili formation eg. Mannocides which compete for mannose binding pockets on FimH subunits. *E. coli* and other Enterobacteriaceae produce and display adhesive amyloid fibers termed curli at the bacterial cell surface, Assembly of curli depends upon atleast six proteins known as CsgA, CsgB, CsgD, CsgE, CsgF, andCsgG. Curlicides inhibit the polymerisation of proteins in curli biosynthesis and inhibit biogenesis of curli eg. pyridones [48].

Modulators of c-di-GMP signaling in bacteria include small molecule inhibitors. It was discovered that the molecule nitric oxide (NO) can induce dispersal of *P. Aeruginosa* biofilms and a combined treatment approach with a NO donor and an antimicrobial agent was suggested to eradicate biofilm infections and S-nitroso-N-acetylpenicillaminewas found as the most potent NO donor [49]. NO-donors cause a down regulation of the synthesis of pyoverdine [50] which is a siderophore responsible for recruitment of essential iron for biofilm formation [51].

Quorum Sensing is a cell to cell communication mechanism in bacteria which controls phenotype manifestations. Signal molecules are constantly produced by each individual bacterium and quorumsensing is activated when the concentration of this molecules reach a threshold. In Gram positive bacteria, the signal molecules consisted in peptides, while Gram negative bacteria use N-acylhomoserine lactones (AHLs). So identification of small molecules that interfere quorum sensing has been identified. Quorum Sensing Inhibitor mechanisms include a. Destruction of signal molecules b. Inhibition of synthesis c.Inhibition of receptor interactions with analogues of signal molecules eg. Peptide homologues, AHL analogues [52].

3.3.5. Nanoparticles

Nanomaterials are defined as the materials with a atleast one of its dimension lesser than 100 nm. Nanoparticles can overcome drug resistance because of their multi functionality as it is not possible to develop multiple mutations simultaneously. Nanoparticles can be organic or inorganic. But metallic nanoparticles have higher loading capacity and stability. Physical and chemical methods are utilised in the synthesis of nanoparticles, Physical methods utilise high energy consumption to trim down bulk materials into fine particles, where as chemical methods utilise synthetic capping, reducing and stabilising agents [53]. Khan *et al*, 2019 reviewed the toxicity associated with chemical methods [54]. Natural resources like microorganisms and plants can be employed as reducing agents in the synthesis of metallic nanoparticles. The main mechanism of nanoparticle synthesis via microbes is by the reductase enzyme or biochemical pathways in bacteria [55]. Microbial based synthesis involves certain drawbacks ie, complexity in culturing techniques and slow processing [56]. Role of plant extract as reducing, stabilising and capping agent is due to carbonyl and hydroxyl groups [57, 58]. Antibacterial mechanisms of nanoparticles include metal ion release, oxidative stress and non oxidative mechanism which helps in affecting cell membrane integrity and permeability. Released metal ions react with cellular constituents leading to cell death.. Reactive oxygen species released in the presence of nanoparticles, inhibits bacterial growth by restricting amino acid synthesis, lipid peroxidation and DNA replication. Reactive oxygen species also alters cell membrane permeability, and cause irreversible membrane damage. [59, 60]. The non oxidative mechanism is by direct interaction of nanoparticles with cell walls through different types of physicochemical interactions. Once the particle gets attached to cell surface, redox reactions takes place to create oxidative stress in bacteria [61]. The Nanoparticles also work as carrier of antibiotics and also in preventing biofilm formation.

4. Conclusion

All renowned antibiotic classes have earned notable resistance. Monotherapy approaches are found to be less effective. Now the focus is on the resistance mechanisms of bacteria. Combinational approach can bypass bacterial resistance mechanisms. Nanotechnology has emerged as an interdisciplinary approach. Preclinical and clinical trials on these different approaches are going on.

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