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Review Article

Antibiotic Adjuvants – A Review Article

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Abstract

Antibiotic resistance (AR) has emerged as a critical global health challenge, affecting both natural and synthetic antibiotics. The search for new, more effective antibiotics is costly and difficult, making alternative strategies, such as antibiotic adjuvants, an important area of focus. This review explores the potential of adjuvants in combating AR. Antibiotic resistance occurs through mechanisms like (i) antibiotic inactivation via enzymatic modification or breakdown, (ii) reduced antibiotic uptake due to increased efflux, and (iii) modification of the antibiotic target site. These mechanisms present opportunities for adjuvant drug development, targeting proteins or enzymes involved in resistance. Recent research highlights broad-spectrum antibiotic adjuvants and hybrid approaches, aiming to inhibit key resistance mechanisms, such as β-lactamase enzymes and efflux pumps, or disrupting bacterial signaling and response systems. Other adjuvants enhance antibiotic uptake, prevent modification of antibiotics or their targets, or target non-essential bacterial processes like cell wall synthesis. While progress is being made, the ongoing race between developing new antibiotic therapies and microorganisms acquiring resistance mechanisms remains a significant challenge.

Keywords*: AR-Antibiotic resistance, antibiotic adjuvants, CDC- Centers for Disease Control and Prevention, MDR-Multi-Drug Resistant, MRSA- Methicillin-Resistant Staphylococcus aureus, PK-Pharmacokinetic, WHO- World Health Organization,*

Introduction: Antibiotic resistance (AR) has now become one of the significant Global Health challenges[1], and the view of AR is no longer being addressed by studying the problem, but it is high time to find solutions. However, long before humans started mass-producing antibiotics, many bacteria evolved to tolerate them and prevent the treatment of infectious diseases[2,3]. An important driver of AR development is likely to be the competition for resources among microorganisms[4,5]. These resources include the natural production of secondary metabolites similar to many commercial antibiotics. "An antibiotic is a chemical substance, produced by microorganisms, which can inhibit the growth of and even destroy bacteria and other microorganisms," the definition provided by S.A. Waksman[6]. While today, "antibiotic" is not limited to a chemical substance produced by microorganisms but a synthetic or natural substance that inhibits or kills bacteria. But the introduction of antibiotics as clinical agents dramatically changed the evolution and spread of AR by providing unprecedented selection pressures[7]. Therefore, scientists need to improve antibiotics regularly. The improvement of antibiotics is mainly based on their mode of action and targets. For example, antibiotics inhibit or kill bacteria by preventing (i) cell-wall biosynthesis; (ii) protein synthesis; (iii) DNA replication and repair; (iv) folic acid metabolism; and/ or disrupting membrane structure[8]. But the recent emergence of multi-drug resistant (MDR) bacteria demands the expedited process of antibiotic improvement. However, a critical point limiting capacity is the flagging investment in research and development of novel antibiotics, mainly due to the low-profit margin.

However, it is crucial to search for more effective antibiotics and develop novel chemical entities with new mechanisms of action. An in-depth investigation of the essential biological and biochemical processes in bacteria and the development of novel scaffolds that target them gives us hope. The availability of genomic

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data has significantly contributed to this progress[9]. Similarly, a great success in minimizing the AR by using an 'antibiotic adjuvant'. These are also known as 'resistance breakers' or 'antibiotic potentiators[10,11]. Antibiotic adjuvants have no or little antibiotic activity. So their mood of action is either by blocking the primary bacterial resistance or by enhancing the antimicrobial action of the drug. Therefore, from the drug discovery point of view, this combined drug therapy has the advantage, and it is unnecessary to go for new target. identifications that are challenging and expensive[8]. This prosperous and successful strategy in combating antibiotic resistance will be the focus of this review.

Antibiotic resistance:

The possible causes of AR are excessive use of antibiotics in animals and humans, easy access to antibiotics, increased international travel, and due to poor sanitation release of non-metabolized antibiotics residues into the environment through manure/faeces[12]. A remarkable amount of antibiotic consumption increases in livestock feed, and it is estimated that the use will increase to 67% in 2030[13]. This uncontrolled use of antibiotics in livestock for infection prevention and growth promotion significantly contributes to the development of AR[14]. However, there might be several physiological and biochemical mechanisms in developing resistance. But, little has been known about these complex mechanisms of emergence and distribution of the resistance[15,16]. After analyzing the available bacterial genome data, more than 20,000 potential resistance genes were identified; however, the functional resistance determinants are fewer[17]. AR was first detected in the early 1960s, among enteric bacteria Escherichia coli, Shigella, and Salmonella. Until then, these resistant strains caused substantial health-economic burdens, mainly in developing countries with common health problems with enteric microbes. But after a decade, it became a global concern when ampicillin-resistant Neisseria gonorrhoeae and Haemophilusinfluenzae were identified and later reported to resist tetracycline and chloramphenicol as well[12,18]. Currently, numerous important organizations, like the World Health Organization (WHO), World Economic Forum and Centers for Disease Control and Prevention (CDC) have declared antibiotic resistance as a 'global public health concern[19,20]. Since then, several social action plans have been announced, including national and international prize announcements to tackle antibiotic resistance[21,22]. In contrast, there are no signs of declining global AR.

Global economy and AR:

Proper estimation of the exact economic impact of AR is still challenging. It requires measuring the disease distribution associated with AR. However, several studies try to illustrate the burden due to AR. In the USA, approximately 100,000 deaths have been recorded yearly due to antibiotic-resistant pathogen-associated hospital-acquired infections[23,24]. In 2006, about 50,000 US citizens died due to sepsis and pneumonia, costing about $8 billion[25]. Patients need to stay long in case of AR pathogen infections, causing an additional 8 million hospital days annually in the US. This extended stay in the hospital costs up to $29,000 per patient treated with an antibiotic-resistant bacterial infection[26]. Another study estimated the global economic burden would be about $120 trillion and about 444 million people would succumb to infections[27].

Causes of antibiotic resistance:

Most of the antibiotics are natural and produced by microbes. Others are semi-synthetic, and few are fully synthetic but have structural similarities to natural antibiotics[28]. Therefore, Various organisms have evolved with defensive mechanisms against them by producing an enzyme that can degrade the antibiotics, changing the target site and inhibiting drug entry or distribution[29].

Extensive diversity in genetic determinants for antibiotic resistance has been revealed by the functional metagenomic analysis[30,31]. Saprophytic bacteria produce various antibiotic molecules that inhibit the growth of other organisms in that environment. But the previous study suggested that antibiotic substances present in low concentrations in the soil; and sub lethal concentrations significantly impact microbial physiology and evolution that may act as effective signaling molecules to induce gene expression[32]. However, the emergence of AR is not happening for natural antibiotics only but also against synthetic antibiotics. Many factors are involved in developing antibiotic resistance; overuse is the principal cause. In 30%–50% of the cases, doctors choose inappropriate antibiotics and therapy

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duration[33,34]. On the other hand, 80% of antibiotics are used in the USA as growth supplements and infection control in animals. In humans, the estimated global antibiotic consumption rate was 14.3 defined daily doses per 1000 populations in 2018, a 46% increase from 2000[35]. Another important driverd of antibiotic resistance includes sanitation and water hygiene systems that allow the release of antibiotic residuals in the environment. In the environment, genetic mutation and the exchange of genes between organisms play an important role in the spread of resistance[29]. Plasmid transmission is the most important way to transfer resistance genes into the host cell[36]. In humans, especially at the community level, resistant pathogens of the family Enterobacteriaceae may transmit through feco–oral route[37]. Community-acquired MRSA is an excellent example of human-to-human resistance transmission due to prolonged hospital stays or unhygienic hospital settings. However, resistance can be transmitted by sexual route too, where drug-resistant N. gonorrhoeae and HIV are examples[38,39]. From animals, mobile genetic elements and resistant bacteria may transmit to humans in different ways[40], environmental transmission is also well-documented through pharmaceutical industry pollution, sewage systems, and waste management procedures[37]. Recently β-lactamases production increased acquired MDR infections leading to third-generation carbapenem and cephalosporin resistance[41]. The important genes responsible for MDR *E. coli* and Salmonella are AmpC, bla-CTXM-15, bla-TEM-1, floR, VIM-1, tetG, NDM-1, and mcr-1[42,43]. These genes can be transferred to other microorganisms using a vector. Normally bacteria use two mechanisms for resistance; (a) intrinsic resistance and (b) acquired resistance (Figure 1)[44]

Intrinsic resistance is known if a bacterium resists a specific antibiotic due to inherent structural or functional properties. Pseudomonas has no susceptible target site for a particular antibiotic and therefore shows an intrinsic resistance mechanism to a broad-spectrum biocide, triclosan[45]. Another example is lipopeptidedaptomycin, an active drug against Gram-positive while useless against Gram-negative bacteria due to intrinsic variation in the cytoplasmic membrane composition[46].

Additionally, some antibacterial compounds cannot cross the outer membrane, which is also considered a way of intrinsic resistance. Here an example is a vancomycin which inhibits peptidoglycan cross linking by targeting d-Ala-d Ala peptides in Gram-positive; while it cannot pass through the outer membrane of Gram-negative bacteria[47]. In case of acquired antibiotic resistance, bacteria use various mechanisms, including antibiotic efflux or poor drug penetration, modification of the antibiotic target site due to genetic mutation or posttranslational target modification, and inactivation of the antibiotic by metabolic modification or hydrolysis[48-50]. An example of this mechanism is plasmid coding colistin-resistant (mcr-1 dependent) genes in *E. coli*.

Antibiotic adjuvants; a way forward:

Due to the current emergency of AR, there is a need to develop alternative approaches to combat resistance; antibiotic adjuvants are receiving increasing attention[51]. The antibiotic adjuvants approach involves the combination of an adjuvant, a non-microbicidal compound, with an antibiotic to increase the antibiotic activity. However, adjuvants typically do not have antibiotic potential when administered alone, contrasting synergistic antibiotic combinations[52]. Combination therapies are challenging for dose optimizing, possibly allowing the continued use of clinically approved antibiotics that may lead to bacterial resistance.

Genotypic antibiotic resistance or intrinsic resistance occurs predominantly by three mechanisms[53]; (i) inactivation of the antibiotic (i.a) enzymatic modification (i.b) enzymatic breakdown, (ii) decreased antibiotic uptake or accumulation within the bacterial cell by increased efflux, (iii) modification of the antibiotic target site resulting reduced affinity (Figure 1). Therefore, proteins or enzymes involved in these resistance mechanisms are potential targets for developing adjuvant drugs.

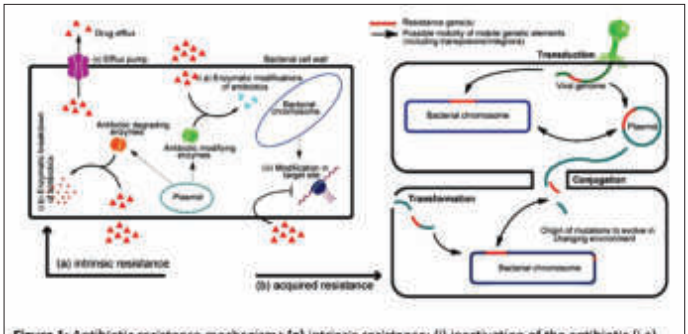

Figure 1: Antibiotic resistance mechanisms **(a)** intrinsic resistance: (i) inactivation of the antibiotic (i.a) enzymatic modification (i.b) enzymatic breakdown, (ii) decreased antibiotic uptake or accumulation within the bacterial cell as a result of increased efflux, (iii) modification of the antibiotic target site

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Inhibition of antibiotic-modifying enzymes:
Antibiotic modifying enzyme production can reduce antibiotic activity, a common mechanism by which bacteria evade the action of these drugs. The modification frequently used by bacteria is hydrolysis; for example, β-lactamase enzymes can hydrolyze the lactam bond of β-lactam antibiotics; macrolide esterases hydrolyze the lactone bond of macrolides[54]. Also, bacteria can modify antibiotics by adding a group to the antibiotics; examples are adding an adenyl, phosphoryl or acetyl group to aminoglycosides by the aminoglycoside-modifying enzymes (AMEs)[55]. Other antibiotic-modifying enzymes include macrolide glycosyltransferases and chloramphenicol acetyltransferases[54]. Redox reactions can also inactivate antibiotics by oxidation of tigecycline by the monooxygenase TetX[56].

β-lactamase inhibitors are classic examples of adjuvants that inhibit modification of the antibiotic[57]. This class of adjuvants are listed in Figure 2[58,59]. Augmentin is a combination of amoxicillin and clavulanic acid that inhibits β-lactamase and cell wall synthesis[60]. β-lactamase inhibitors sulbactam and tazobactam are specific for class A β-lactamases but not against class C. Therefore, recently non-β-lactam-derived β-lactaminhibitors adjuvants of the di-aza-bi-cyclo-octanes (DBO) class are in focus. They are active against the class C β-lactamases[61]. Avibactam was approved in 2015; a member of this class which is susceptible to hydrolysis upon binding to the β-lactamase, as the de-acylation mechanism, releases the intact inhibitor[62]. Another member of the DBO class of β-lactamase inhibitors is Relebactam (MK-7665) in combination with imipenem/cilastatin. Other member of this class includes the 6-methylidene-penem compound BLI-489 and Tri-cyclic-carbapenem LK-157[63,64].

Another class of adjuvants is the boronic acid class of β-lactamase inhibitors, including Vaborbactam; in combination with biapenem, Vaborbactam can inhibit class A and C β-lactamase[65]. Vaborbactam can also be used with meropenem against carbapenemases-producing Enterobacteriaceae[66,67]. β-Lactamase inhibitors that are active against metallo-β-lactamases include the fumarate derivative ME1071 which significantly enhances the activity of biapenem against Pseudomonas aeruginosa[63]. The triple combination of Clavulanic acid, bridged monobactam BAL29880 and siderophore monobactam BAL19764 is also used to inhibit metalo- β-lactamase producing Enterobac teriaceae[68]. Also, the bisthiazolidine class of compounds used to inhibit metalo- β-lactamase-producing Escherichia coli[69]. In 2014, Aspergillomarasmine A used as an inhibitor of the mammalian metalloenzymes angiotensin- converting enzyme and endothelin-converting enzyme, which acts as promising adjuvants against metalo-β-lactamase-producing bacteria[70] (Figure 2).

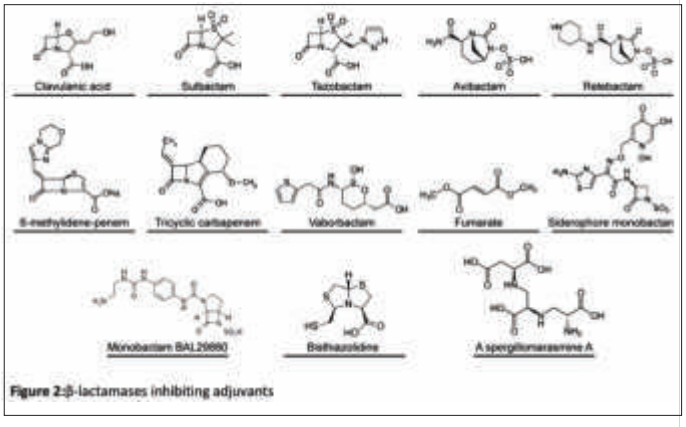

Figure 2:β-lactamases inhibiting adjuvants

Although, the development of adjuvants that inhibit modification of other antibiotics classes have also been investigated71 (Figure 3).

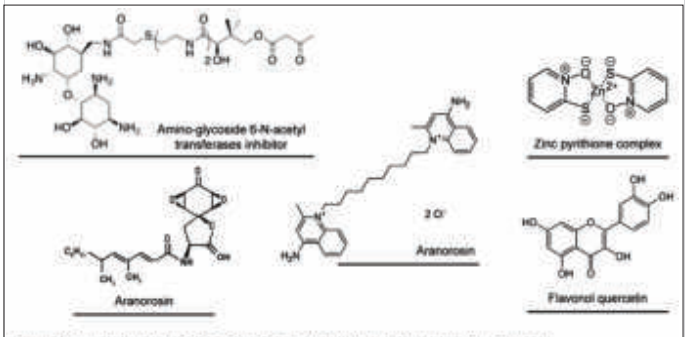

Figure 3: Adjuvants that inhibit antibiotic-modifying enzymes other than β-lactamases

AMEs are mainly responsible for aminoglycoside antibiotic resistance by adding a functional group that interrupts the interaction of the antibiotic with the rRNA target. Nucleotidyl-tranferases, phosphor-transferases, and acetyl-transferases are three AMEs that modify both hydroxyl and aminegroups[55]. Inhibitors of these three enzymes are prospective adjuvants for treating infections caused by Gram-negative bacteria[72]. Aminoglycoside 6-N-acetyl-transferases can transfer an acetyl group from acetyl-coenzyme A to the amino group at the 6 positions of the aminoglycoside.

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Aminoglycoside 6-N-acetyl-transferases inhibitor acted synergistically with Kanamycin against Enterococcus faecium[73]. The zinc pyrithione complex also suppressed amikacin resistance *E. coli* that can produce aminoglycoside 6-N-acetyl-transferases[74]. It was also effective against amikacin andtobramycin resistance Gram-negative bacterial species, including Enterobacter cloacae and K. pneumoniae[75]. Similarly, a copper pyrithione complex can suppress amikacin resistance in K. pneumoniae[76].

A study identified 14 bacterial kinases involved in antibiotic resistance, where flavonol quercetin can inhibit 12 of them, including all amino- glycoside-phospho-transferases. This adjuvant significantly increased aminoglycoside antibiotics activity on amino-glycoside-phospho-transferases producing *E.coli*[77]. Another adjuvant, aranorosin has been reported to active against methicillin-resistant Staphylococcus aureus (MRSA)[78]. Mycobacterium species use mycothiol to maintain an intracellular reducing environment and detoxify xenobiotics[79]. Dequalinium is an inhibitor of mycothiol biosynthetic enzyme MshC[80], and can enhance spectinomycin' santibiotic activity against Mycobact- erium smegmatis[81].

Inhibition of target alteration:

Bacteria may also alter the target of the antibiotic. But only a few adjuvants successfully targeted this resistance mechanism[71]. The ErmC methyl-transferase enzymes catalyze adenine methylation in bacterial 23S rRNA and develop resistance against macrolide- lincosamide- streptogramin-B (MLS) classes of antibiotics[82]. ErmC inhibitor exhibited synergistic activity with azithromycin against Enterococcus faecalisand S. aureus and erythromycin against *E. coli* strains expressing ErmC methyl-transferase enzymes[83].

Inhibition of efflux:

Membrane-bound efflux proteins pump toxic agents; therefore, bacteria also use these efflux proteins to pump out antibiotics. These pumps are specific for one substrate or class. However, these can also be effective for multiple antibiotics classes (Table 1), including clinically relevant Mex and AcrAB-TolC pumps. Additionally, efflux pumps can synergi stically act with other resistance mechanisms, such as Gram-negative bacteria's outer membrane permeability barrier, exacerbating resistance[84].

Efflux Pumps	Bacteria	Antibiotic Resistance	References
AcrAB-TolC	*Salmonella enterica*	Quinolones, Chloramphenicolflorfenicol, Tetracyclines	[85]
AcrAB	*Shigellaflexneri, Escherichia coli*	Fluroquinolone	[86]
LpeAB	*Legionella pneumophila*	Macrolides	[87]
MexAB-OprM	*Pseudomonas aeruginosa*	Carbapenem, Fluroquinolones	[85, 86]
MexEF-OprN	*Pseudomonas aeruginosa*	Quinolones, Chloramphenicol, Trimethoprim. Imipenem	[88]
MdfA	*Escherichia coli*	Aminoglycosides, Neomycin, Kanamycin	[89]
MtrCDE	*Neisseria gonorrhoeae*	Penicillin	[90]
NorA	*Staphylococcus aureus*	Fluroquinolones	[91]

S. aureus can express more than 15 efflux pumps; some are chromosomally encoded and some from plasmid[92]. NorA efflux pump plays a role in fluoroquinolone antibiotics resistance and also for at least 10% antibacterial resistance in MRSA strains[93]. The plant alkaloid reserpine (Figure 4) can inhibit NorA-mediated drug efflux; additionally, reserpine increases the effect of ciprofloxacin and bactericidal activity on S. aureus.

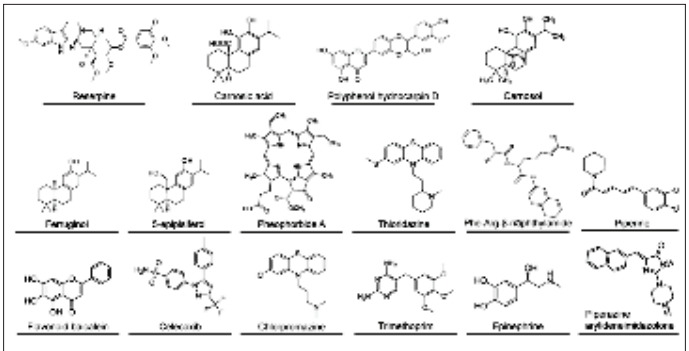

Figure 4: Inhibitors of efflux pumps in Gram-negative bacteria

Due to the neurotoxicity effect, reserpine cannot be used in a clinical setting. Other phytochemicals, including carnosol and carnosic acid, also inhibit several efflux pumps of S. aureus; i.e. TetA and MsrA efflux pumps involved in tetracycline and erythromycin resistance[93]. Abietanesferruginol, 5-epipisiferol, chlorophyll metabolitep heophor bideA, polyphenol hydnocarpin D, and flavonoid baicalein (Figure 4) are also studied as NorA inhibitors[71]. Table 1: Examples efflux pumps and resistance phenotype in bacteria. Celecoxib is a NorA inhibitor that can suppresses drug resistance in the cancer cell with multiple antibiotic classes, including ampicillin, chloramphenicol, kanamycin, and ciprofloxacin[94]. Thioridazine has modest antibiotic

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activity and can inhibit both, efflux-mediated and non-mediated resistance mechanisms[95]. MdeA efflux pump is responsible for resistance to several antibiotics, including mupirocin and novobiocins; alkaloid piperine can inhibit MdeA and NorA in S. aureus[92].

Different efflux pumps have been described in other Gram-negative bacteria, such as MexEF-OprN, MexAB-OprM, MexCD-OprJ, and MexXY-OprM pumps of P. aeruginosa. Phe-Arg-β-naphthylamide (PAβN) is an inhibitor of these four efflux pumps[96]. Another multi-drug resistance efflux pump in Enterobacteriaceae is AcrAB-TolC, which is regulated by the transcriptional activator RamA encoded by a gene of the samename, ramA[97,98]. PAβN upregulates ramA gene and interrupts AcrAB-TolC production, while thioridazine, phenothiazine, trimethoprim, and epinephrine chlorpromazine inhibit the AcrAB-TolC efflux system and increase susceptibility to several antibiotics, including norfloxacin, nalidixic acid, chloramphenicol, tetracycline, and ciprofloxacin. However, phenothiazines affect efflux-related gene expression and suppress resistance[98,99]. Another adjuvant piperazinearylideneimidazolone can inhibit efflux by overexpressing acrAB in *E. coli* and increase susceptibility to clarithromycin, levofloxacin, linezolid, and oxacillin[97].

Enhancement of antibiotic uptake:

Several antibiotic targets are located within the cytoplasm; therefore, they must cross bacterial cell walls. The Gram-positive cell wall is relatively permeable than Gram-negative. Several compounds can destabilize the Gram-negative outer membrane and increase antibiotic uptake. Polymyxin B nonapeptide (PMBN) (Figure 5), increases the susceptibility of Gram-negative bacteria, including P. aeruginosa and K. pneumoniae tonovobiocin, fusidic acid and erythromycin [100].

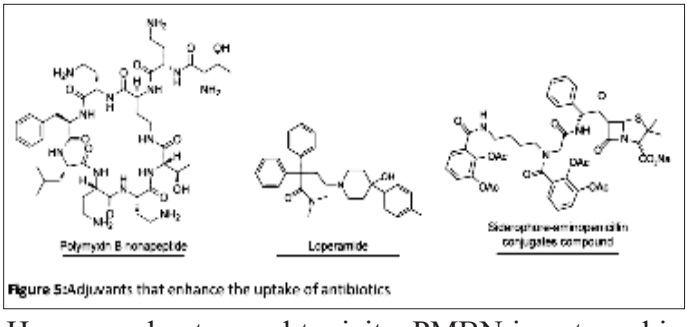

Figure 5: Adjuvants that enhance the uptake of antibiotics

However, due to renal toxicity, PMBN is not used in the clinical sector; it requires developing second-generation analogs with reduced toxicity[101]. Adjuvant loperamide can increase tetracycline uptake in Gram-negative bacteria, including E. coli, A. baumannii, P. aeruginosa, Salmonella enterica, and K. pneumoniae[102]. Pathogenic bacteria use siderophore-specific receptors for ironentry into the cell. Siderophore-aminopenicillin conjugates allow antibiotic uptake using the iron channel and are active against carbapenem-resistant isolates of S. maltophilia and P. aeruginosa[103].

Interfering with signaling systems

Interfering with the ability of the bacteria to "switch on" resistance machinery is an alternative method against AR. Bacteria use various pathways to sense antibiotics and activate or upregulate the production of the proteins required for resistance. For example, MRSA can detect β-lactam antibiotics by the MecR1 and BlaR1 sensor systems and then subsequently initiate the encoding of β-lactamase and penicillin-binding protein 2a (PBP2a) to get resistance. Mammalian serine/threonine kinase inhibitors (Figure 6) reduce the phosphorylation of BlaR1 in the presence of penicillin[104].

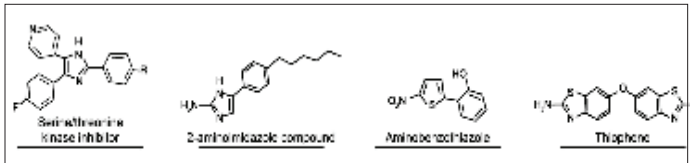

Figure 6: Inhibitors of bacterial signaling systems involved in antibiotic resistance

A prominent signaling and regulatory system is the two-component system (TCS), which controls the response to external stimuli and stresses. TCS can control sporulation, biofilm formation, competence, pathogenesis, and antibiotic resistance across multiple bacterial species[105,106]. TCS depends on histidine kinase and can control gene expression in response to environmental change by phosphatases and dephosphorylate activity[105]. VraRS system in MRSA is a good example of TCS that allow antibiotic resistance[107]. VraRS senses cell wall damage and coordinates a response involving numerous genes activation for cell wall synthesis. Multiple TCSs are responsible for the variation in β-lactam resistance in MRSA, which can be inhibited by 2- aminoimidazole compounds derived from marine natural products[108]. Aminobenzothiazole and thiophene (Figure 6) exhibited moderate antibiotic activity against *E. coli* and Bacillus subtilis by inactivating histidine kinases[109].

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Targeting non-essential steps in cell wall synthesis

There are several proteins and enzymes involved in bacterial cell wall synthesis. In S. aureus, deletion of some peptidoglycan synthesis genes does not affect cell growth or morphology but increases susceptibility to cell wall-acting antibiotics[110]. These types of non-essential genes are ideal targets for adjuvants. In the Gram-positive cell wall, glycophosphate polymer wall teichoic acid (WTA) has no function for survival; however, inactivation or alteration of WTA in MRSA increases susceptibility to β-lactam antibiotics[111]. TarO gene-encoded enzyme involved in the early stages of WTA synthesis. A natural product, tunicamycin (Figure 7), inhibits the TarO, and peptidoglycan synthesis enzyme MraY makes S. aureus susceptible to β-lactam antibiotics[112].

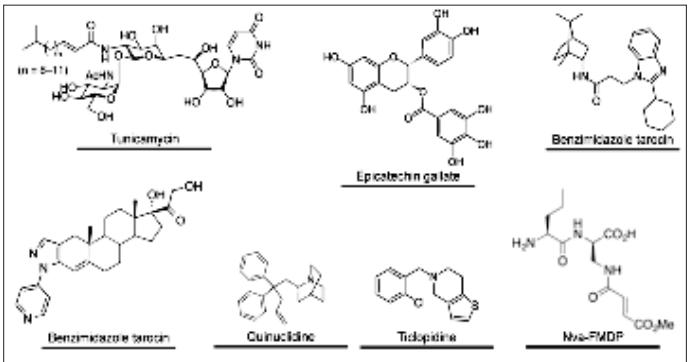

Figure 7: Adjuvants that target cell wall synthesis

However, due to toxicity, tunicamycin cannot be used clinically. Intoxicticlopidine and benzimidaz oletarocin B are used with cefuroxime against wild-type MRSA[113]. The highly conserved cytoskeletal protein FtsZ plays an essential role in cell division[114]. Inhibition of FtsZ using thiazolo-pyridine PC190723, enhances the activity of cell-wall-acting antibiotics at sub-microbicidal concentrations[115] Another FtsZ inhibitor is quinuclidine[116], used with ceftriaxone against Gram-negative pathogens, including P. aeruginosa, K. pneumonia, *E. coli*, and A. baumannii[117]. Nva-FMDP (Figure 7) is an inhibitor of the enzyme encoded by GlmS gene, which is involved in the synthesis of the peptidoglycan precursor[118].

Enhancing host defense

Most recently, scientists are not only focusing on the conventional direct pathogen-target approach. The human innate immune system is the best defense against MDR bacterial infections. Thus enhancing host cell responses for pathogen eradication is a new approach. An example of 'host defense targeted' therapeutic is using immunomodulatory peptides such as LL-37. LL-37 up regulate neutrophil and down regulate pro-inflammatory cytokines and IFN-c, thus enhance the antibacterial activity of the innate immune system[119]. Also, most recently, lactoferritin derivative hLF1-11, displayed antibacterial activity in a rabbit osteomyelitis infection model[120]. Interestingly, some molecules possess immunomodulatory properties and direct antibacterial activity. For example, non-peptide-based amphiphilic tobramycin analogs can boost the immune response by recruiting neutrophils required to resolve bacterial pathogens. Moreover, amphiphilic tobramycin analogs can selectively control inflammatory responses[121].

New research possibilities:

Broad-spectrum antibiotic adjuvants:

Broad-spectrum antibiotics have disadvantages, such as triggering hyper-inflammatory responses, disrupting the beneficial micro biome, and developing AR. Therefore we need to select pathogen-specific antibiotics[122]. But in the clinical sector, specific pathogen identification and antibiotic susceptibility test may not be possible due to medical emergencies. In this case, broad-spectrum antibiotic adjuvants could be a possible solution, hanse they have little or no antibiotic activity and might have no evolutionary pressure for AR development. However, most antibiotic adjuvants are species-specific due to their mode of action. This strategy requires further investigations with a greater understanding of bacteria's universal resistance and adjuvant mechanism.

Hybrids approach for antibiotic-adjuvant:

Although many adjuvants showed an effective result in in-vitro but failed in in-vivo treatment, mainly due to different pharmacological properties, such as tissue distribution and penetration. The hybrid approach for antibiotic-adjuvant offers an alternative to avoid this challenge. An example of such strategies is using amino-glycoside-tri-cosan analog combinations to enhance antibacterial activity against neomycin-resistant P. aeruginosa[123]. Notably, antibiotic-adjuvant conjugates may also encounter pharmacokinetic (PK) problems of their molecular size for tissue uptake and distribution. Recently,

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tobramycin-based hybrids have been systematically reviewed[124]. However, further study on molecular complexity and intractable chemical synthesis is required to establish the benefit of the hybrids approach.

Conclusions:

There is a race between humans and microorganisms for developing new drugs with antibiotic activity versus acquiring resistance mechanisms. The causes of AR are complex and involve not only the selective pressure exerted by the overuse of antibiotics but also by environmental pollution with disinfectants, pollutants, and heavy metals; as well as intrinsic factors natural to microorganisms, such as horizontal gene transfers. Understanding the molecular pathways involved in drug uptake is important for developing and discovering new antibiotic adjuvants against pathogens. The use of antibiotic adjuvants is an important strategy to restore and preserve the activity of available antibiotics. Also, developing adjuvants is more cost-effective than developing or discovering new broad-spectram antibiotics. This study reviewed the literature on different ways to develop AR and prospective adjuvants with the mode of action and their antibiotic combination. Furthermore, several approaches to adjuvants have been discussed, from the well-known and clinically validated approach of inhibiting β-lactamase enzymes and efflux pumps to more indirect approaches, such as inhibiting bacterial signaling and response systems that mediate AR. Adjuvants that act by increasing cellular uptake of antibiotics, adjuvants that inhibit modification of the antibiotic or its target, and finally, the identification of adjuvants that act upon less obvious targets, such as non-essential steps in bacterial cell wall synthesis, are also discussed.

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