Antibiotic Adjuvants in Combating Antibiotic Resistance

By:

Nazoa Shimin Tui

18276005

A thesis submitted to the Department of Mathematics and Natural Sciences in partial fulfillment of the requirements for the degree of

Master of Science in Biotechnology

Department of Mathematics and Natural Sciences

BRAC University

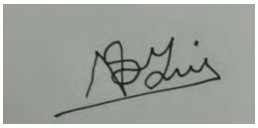
January 2023

Declaration

It is hereby declared that

1. The thesis submitted is my/our own original work (review article) while completing Maters of Science degree at BRAC University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. I/We have acknowledged all main sources of help.

Student's Full Name & Signature:



Nazoa Shimin Tui

_____

Student ID: 18276005

Approval

The thesis titled “Antibiotic adjuvants in combating antibiotic resistance” submitted by

Nazoa Shimin Tui (18276005),

has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Masters in Biotechnology on 26.01.2023.

Examining Committee:

Supervisor:

(Member)

_____

Iftekhar Bin Naser, PhD

Associate Professor,

Department of Mathematics and Natural Sciences,

BRAC University

Program Director:

(Member)

_____

Dr. Munima Haque, PhD

Associate Professor,

Department of Mathematics and Natural Sciences

BRAC University

External Expert Examiner:

(Member) _____

Departmental Head:

(Chair) _____

A F M Yusuf Haider, PhD

Chairman and Professor,

Department of Mathematics and Natural Sciences,

BRAC University.

Ethics Statement

This material is an original work (review article), which has not been previously published elsewhere. It is my own research and analysis in a truthful and complete manner. The paper properly credits all the sources used (correct citation).

Abstract

Antimicrobial resistance (AMR) has now become one of the significant global health challenges and are not limited to natural antibiotics but also for synthetic antibiotics. Therefore, it is crucial to search for more effective antibiotics and develop novel chemical entities with new mechanisms of action. But the process is challenging and expensive. Antibiotics adjuvants gives us hope in combat with AMR. This prosperous and successful strategy in combating antibiotic resistance will be the focus of this review. Genotypic antibiotic resistance or intrinsic resistance occurs predominantly by three mechanisms (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. Therefore, proteins or enzymes involved in these resistance mechanisms are potential targets for developing adjuvant drugs. Another approach is enhancing host cell responses using therapeutic for pathogen eradication. Current research with broad-spectrum antibiotic adjuvants and hybrids approach for antibiotic-adjuvant also being studied. However, there is a race between humans and microorganisms for developing new drugs with antibiotic activity versus acquiring resistance mechanisms. In the current study, 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 AMR. 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.

This work is dedicated to

My Dear Parents

Acknowledgement

First of all, I wish to declare my humble gratitude to Almighty Allah, who has bestowed the gift of outmost mercy, and given me the strength, patience and understanding required to complete this work amid this Covid- 19 situation.

I convey my special thanks to Professor A. F. M. Yusuf Haider, PhD, Chairman, Department of Mathematics and Natural Sciences, BRAC University, who always has tremendous support for every student. I express my gratitude to my supervisor, Iftekhar Bin Naser, PhD, Assistant Professor, Department of Mathematics and Natural Sciences, BRAC University, for his encouragement, continuous guidelines and having faith on me for this research . I am indebted to all the extraordinary people of the Life Science Laboratories, BRAC University. Without their all-time support this would not be complete. I would like to specially recognize Asma Afzal, Lab officer. I would like to express my deepest respect to Mahboob Hossain, PhD, Professor, Department of Mathematics and Natural Sciences, BRAC University for always being a great inspirer.I would also like to express my respect to Romana Siddique, Lecturer, Department of Mathematics and Natural Sciences, BRAC University for her cordial support.

I sincerely thank my parents who trusted me and are always there for me. This journey would be incomplete without some dear friends; Salman Haque, Samiha Haque farin were my greatest supports while I did this work. I am grateful to all of these people.

Sincerely,

Nazoa Shimin Tui,

Department of Mathematics and Natural Sciences, BRAC University

Brief contents

List of figures

List of table

List of acronyms

AMEs	Aminoglycoside-Modifying Enzymes
AMR	Antimicrobial resistance
CDC	Centers for Disease Control and Prevention
DBO	Di-aza-Bi-cyclo-Octanes
MDR	Multi-Drug Resistant
MLS	Macrolide-LincosamideStreptogramin-B
MRSA	Methicillin-Resistant Staphylococcus aureus
PAβN	Phe-Arg-β-naphthylamide
PK	pharmacokinetic
PMBN	Polymyxin B nonapeptide
TCS	Two-component system
WHO	World Health Organization
WTA	Wall teichoic acid

Introduction

Antimicrobial resistance (AMR) has now become one of the significant Global Health challenges (Berendonk et al., 2015), and the view of AMR 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 (Bhullar et al., 2012; D'Costa et al., 2011). An important driver of AMR development is likely to be the competition for resources among microorganisms(Allen et al., 2010; Davies & Davies, 2010). 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 (Waksman, 1947). 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 AMR by providing unprecedented selection pressures (Alcock et al., 2020). 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 (González-Bello, 2017). 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 data has significantly contributed to this progress (Kostyanev et al., 2016). Similarly, a great success in minimizing the AMR by using an 'antibiotic adjuvant'. These are also known as 'resistance breakers' or 'antibiotic potentiators' (Bush, 2015a; Gill et al., 2015). 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 (González-Bello, 2017). This prosperous and successful strategy in combating antibiotic resistance will be the focus of this review.

Antimicrobial resistance (AMR)

The possible causes of AMR 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 (Aslam et al., 2018). A remarkable amount of antimicrobial consumption increases in livestock feed, and it is estimated that the use will increase to 67% in 2030 (Tiseo et al., 2020). This uncontrolled use of antimicrobials in livestock for infection prevention and growth promotion significantly contributes to the development of AMR (Pokharel et al., 2020). 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 (Baker et al., 2018; Lesho & Laguio-Vila, 2019). After analyzing the available bacterial genome data, more than 20,000 potential resistance genes were identified; however, the functional resistance determinants are fewer (Ebmeyer et al., 2021).

AMR 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 *Haemophilus influenzae* were identified and later reported to resist tetracycline and chloramphenicol as well (Aslam et al., 2018; Talebi Bezmin Abadi et al., 2019). 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' (Hoffman et al., 2015; Spellberg et al., 2016). Since then, several social action plans have been announced, including national and international prize announcements to tackle antibiotic resistance (Landers & Kavanagh, 2016; Payne et al., 2015). In contrast, there are no signs of declining global AMR.

Global economy and AMR

Proper estimation of the exact economic impact of AMR is still challenging. It requires measuring the disease distribution associated with AMR. However, several studies try to illustrate the burden due to AMR. In the USA, approximately 100,000 deaths have been recorded yearly due to antibiotic-resistant pathogen-associated hospital-acquired infections

(Umscheid et al., 2011; Zimlichman et al., 2013). In 2006, about 50,000 US citizens died due to sepsis and pneumonia, costing about $8 billion (America, 2011). Patients need to stay long in case of AMR 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 (Ventola, 2015). Another study estimated the global economic burden would be about $120 trillion and about 444 million people would succumb to infections (Ahmad & Khan, 2019).

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 (Wright, 2014). Therefore, Various organisms have evolved with defensive mechanisms against them by producing an enzyme that can degrade the antimicrobials, changing the target site and inhibiting drug entry or distribution (Holmes et al., 2016). Extensive diversity in genetic determinants for antibiotic resistance has been revealed by the functional metagenomic analysis (McGarvey et al., 2012; Nielsen et al., 2022). Saprophytic bacteria produce various antimicrobial molecules that inhibit the growth of other organisms in that environment. But the previous study suggested that antimicrobial substances present in low concentrations in the soil; and sublethal concentrations significantly impact microbial physiology and evolution that may act as effective signalling molecules to induce gene expression (Andersson & Hughes, 2014). However, the emergence of AMR is not happening for natural antimicrobials only but also against synthetic antimicrobials.

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 duration (Durkin et al., 2018; Schmidt et al., 2021). 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 (Klein et al., 2021). Another important drivers of antibiotic resistance include 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 (Holmes et al., 2016). Plasmid transmission is the most important way to transfer resistance

genes into the host cell (Munita & Arias, 2016). In humans, especially at the community level, resistant pathogens of the family *Enterobacteriaceae* may transmit through feco–oral route (Wellington et al., 2013). 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 (Lewis, 2013; Rahman et al., 2022). From animals, mobile genetic elements and resistant bacteria may transmit to humans in different ways (Hernando-Amado et al., 2019); environmental transmission is also well-documented through pharmaceutical industry pollution, sewage systems, and waste management procedures (Wellington et al., 2013).

Recently β-lactamases production increased acquired MDR infections leading to third-generation carbapenem and cephalosporin resistance (Blair et al., 2015). 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 (He et al., 2020; Pazda et al., 2019). 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)**(Lynch III et al., 2013). 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 (Zhu et al., 2010). Another example is lipopeptide daptomycin, an active drug against Gram-positive while useless against Gram-negative bacteria due to intrinsic variation in the cytoplasmic membrane composition (Randall et al., 2013).

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 crosslinking by targeting d-Ala-dAla peptides in Gram-positive; while it cannot pass through the outer membrane of Gram-negative bacteria (Blake & O'Neill, 2013). 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 (Girlich et al., 2020; MacLean & San

Millan, 2019; McInnes et al., 2020). An example of this mechanism is plasmid coding colistin-resistant (mcr-1 dependent) genes in *E. coli*.

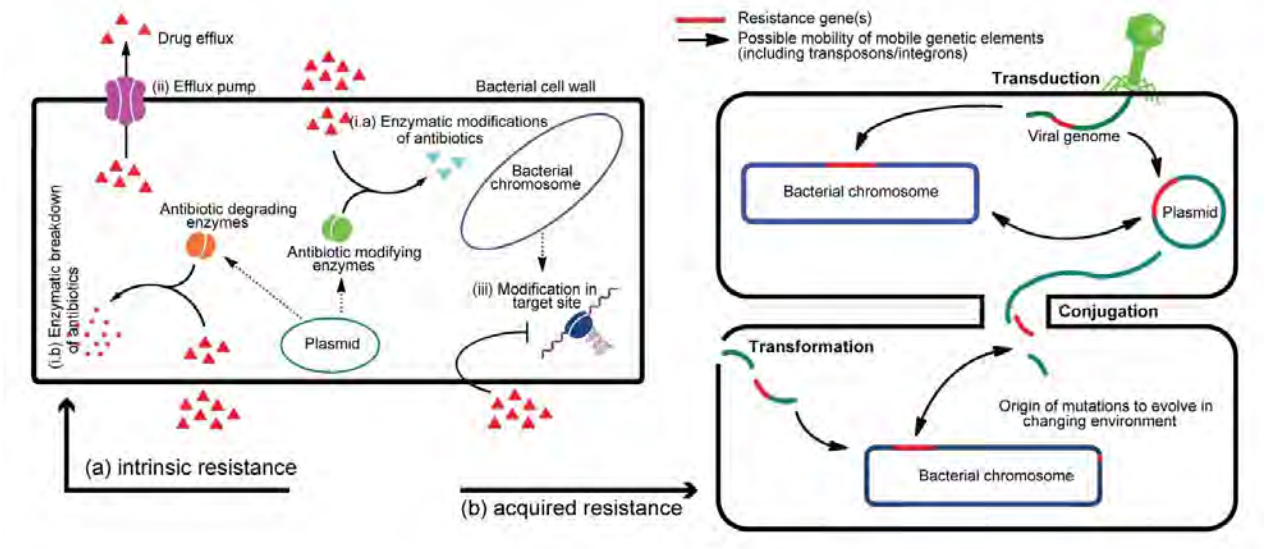


Figure 1: Antibiotic resistance mechanisms **(a)** intrinsic resistance: (i) inactivation of the antibiotic (i.a) enzymatic modification (i.b) enzymatic breakdown, (ii) decreased antibiotic uptake or accumulation within the bacterial cell as a result of increased efflux, (iii) modification of the antibiotic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

Antibiotic adjuvants; a way forward

Due to the current emergency of AMR, there is a need to develop alternative approaches to combat resistance; antibiotic adjuvants are receiving increasing attention (Sharma et al., 2021). The antibiotic adjuvants approach involves the combination of an adjuvant, a non-microbicidal compound, with an antibiotic to increase the antimicrobial activity. However, adjuvants typically do not have antimicrobial potential when administered alone, contrasting synergistic antibiotic combinations (Roemer & Boone, 2013). 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 (Walsh, 2000); (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.

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 (Wright, 2005). 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) (Ramirez & Tolmasky, 2010). Other antibiotic-modifying enzymes include macrolide glycosyltransferases and chloramphenicol acetyltransferases (Wright, 2005). Redox reactions can also inactivate antibiotics by oxidation of tigecycline by the monooxygenase TetX (Volkers et al., 2011).

β-lactamase inhibitors are classic examples of adjuvants that inhibit modification of the antibiotic (Jovetic et al., 2010). This class of adjuvants are listed in **Figure 2**(Bush, 2015b; Papp-Wallace & Bonomo, 2016). Augmentin is a combination of amoxicillin and clavulanic acid that inhibits β-lactamase and cell wall synthesis (Ball, 2007). 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-lactam inhibitors adjuvants of the di-aza-bi-cyclo-octanes (DBO) class are in focus. They are active against the class C β-lactamases (Shlaes, 2013). 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 (Ehmann et al., 2012). 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 (Bassetti et al., 2011; Paukner et al., 2009).

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-lactamases (Livermore & Mushtaq, 2013). Vaborbactam can also be used with meropenem against carbapenemases-producing *Enterobacteriaceae*(Griffith et al., 2016; Lapuebla et al., 2015). 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*(Bassetti et al., 2011). The triple combination of Clavulanic acid, bridged monobactam BAL29880 and siderophore monobactam BAL19764 is also used to inhibit metalo- β-lactamase producing *Enterobacteriaceae*(Page et al., 2011). Also, the bisthiazolidine class of compounds used to inhibit metalo- β-lactamase-producing *Escherichia coli*(Hinchliffe et al., 2016). 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 (King et al., 2014) (**Figure 2**).

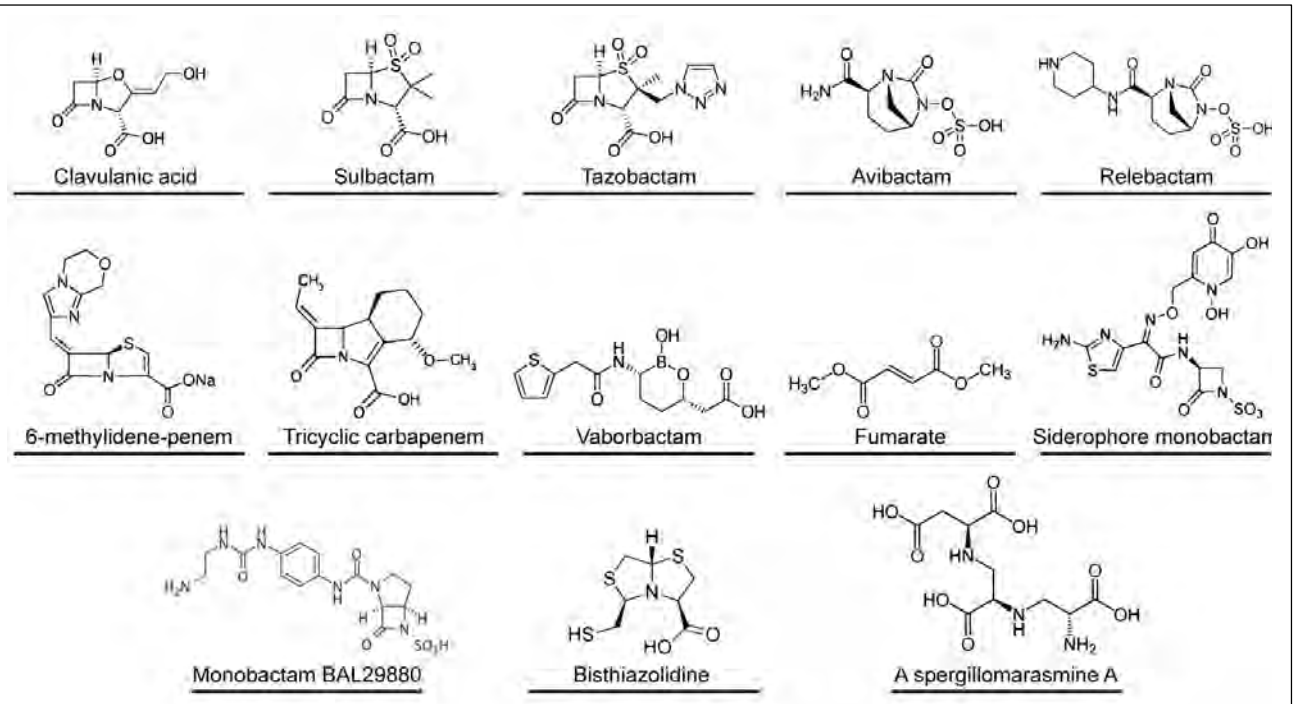


Figure 2:β-lactamases inhibiting adjuvants

Although, the development of adjuvants that inhibit modification of other antibiotics classes have also been investigated (Melander & Melander, 2017) (**Figure 3**). 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 amine groups (Ramirez & Tolmasky, 2010). Inhibitors of these three enzymes are prospective adjuvants for treating infections caused by Gram-negative bacteria (Labby & Garneau-Tsodikova, 2013). 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. Aminoglycoside 6-N-acetyl-transferases inhibitor acted synergistically with Kanamycin against *Enterococcus faecium*(Gao et al., 2006). The zinc pyrithione complex also suppressed amikacin resistance *E. coli* that can produce aminoglycoside 6-N-acetyl-transferases (Lin et al., 2014). It was also effective against amikacin and tobramycin resistance Gram-negative bacterial species, including *Enterobacter cloacae* and *K. pneumoniae*(Y. Li et al., 2015). Similarly, a copper pyrithione complex can suppress amikacin resistance in *K. pneumoniae*(Chiem et al., 2015).

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*(Shakya et al., 2011). Another adjuvant, aranorosin has been reported to active against methicillin-resistant *Staphylococcus aureus* (MRSA) (Suga et al., 2012). *Mycobacterium* species use mycothiol to maintain an intracellular reducing environment and detoxify xenobiotics (Hernick, 2013). Dequalinium is an inhibitor of mycothiol biosynthetic enzyme MshC (Gutierrez-Lugo et al., 2009), and can enhance spectinomycin's antimicrobial activity against *Mycobacterium smegmatis*(Ramón-García et al., 2011).

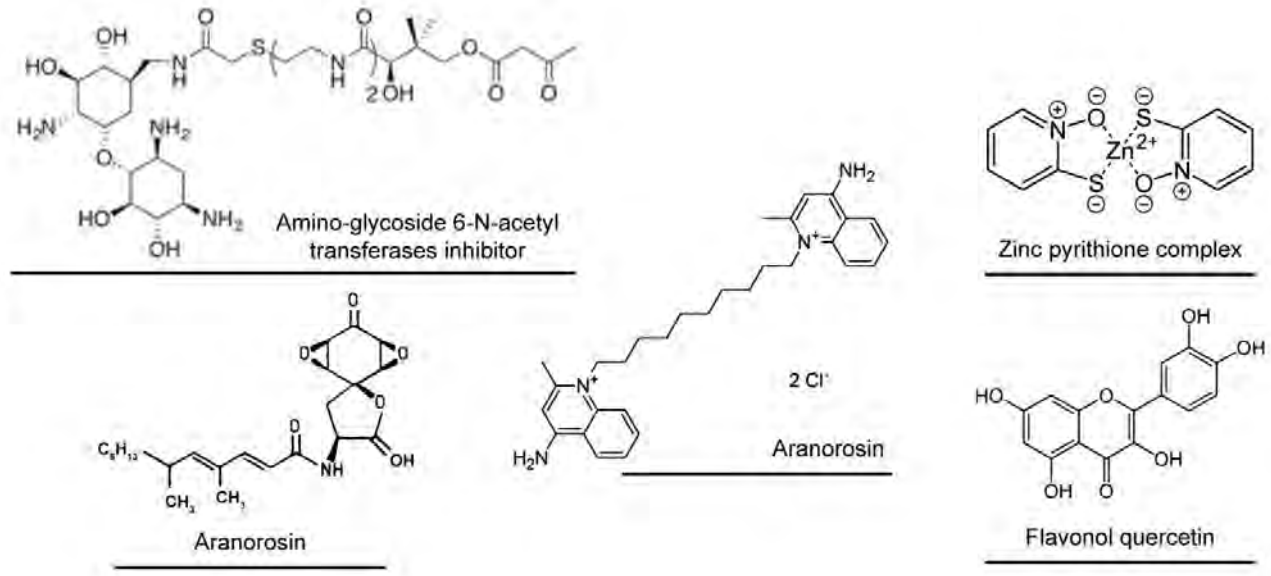


Figure 3: Adjuvants that inhibit antibiotic-modifying enzymes other than β-lactamases

Inhibition of target alteration

Bacteria may also alter the target of the antibiotic. But only a few adjuvants successfully targeted this resistance mechanism (Melander & Melander, 2017). The ErmC methyl-transferase enzymes catalyze adenine methylation in bacterial 23S rRNA and develop resistance against macrolide-lincosamide-streptogramin-B (MLS) classes of antibiotics (Pieren & Tigges, 2012). ErmC inhibitor exhibited synergistic activity with azithromycin against *Enterococcus faecalis* and *S. aureus* and erythromycin against *E. coli* strains expressing ErmC methyl-transferase enzymes (Feder et al., 2008).

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 synergistically act with other resistance mechanisms, such as Gram-negative bacteria's outer membrane permeability barrier, exacerbating resistance (X.-Z. Li et al., 2015).

Table 1: Examples efflux pumps and resistance phenotype in bacteria.

Efflux Pumps	**Bacteria**	**Antibiotic Resistance**	**References**
AcrAB-TolC	*Salmonella enterica*	Quinolones, Chloramphenicolflorfenicol, Tetracyclines	(Pan et al., 2016)
AcrAB	*Shigella flexneri, Escherichia coli*	Fluroquinolone	(Adabi et al., 2015)
LpeAB	*Legionella pneumophila*	Macrolides	(Massip et al., 2017)
MexAB-OprM	*Pseudomonas aeruginosa*	Carbapenem, Fluroquinolones	(Adabi et al., 2015; Pan et al., 2016)
MexEF-OprN	*Pseudomonas aeruginosa*	Quinolones, Chloramphenicol, Trimethoprim, Imipenem	(Ko¨ hler et al., 1997)
MdfA	*Escherichia coli*	Aminoglycosides, Neomycin, Kanamycin	(Putman et al., 2000)
MtrCDE	*Neisseria gonorrhoeae*	Penicillin	(Poole, 2007)
NorA	*Staphylococcus aureus*	Fluroquinolones	(Schmitz et al., 1998)

S. aureus can express more than 15 efflux pumps; some are chromosomally encoded and some from plasmid (Jang, 2016). NorA efflux pump plays a role in fluoroquinolone antibiotics resistance and also for at least 10% antibacterial resistance in MRSA strains (Abreu et al., 2012). 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.* Due to the neurotoxicity effect, reserpine can not 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 (Abreu et al., 2012). Abietanes ferruginol, 5-epipisiferol, chlorophyll metabolite pheophorbide A, polyphenol hydnocarpin D, and flavonoid baicalein (**Figure 4**) are also studied as NorA inhibitors (Melander & Melander, 2017).

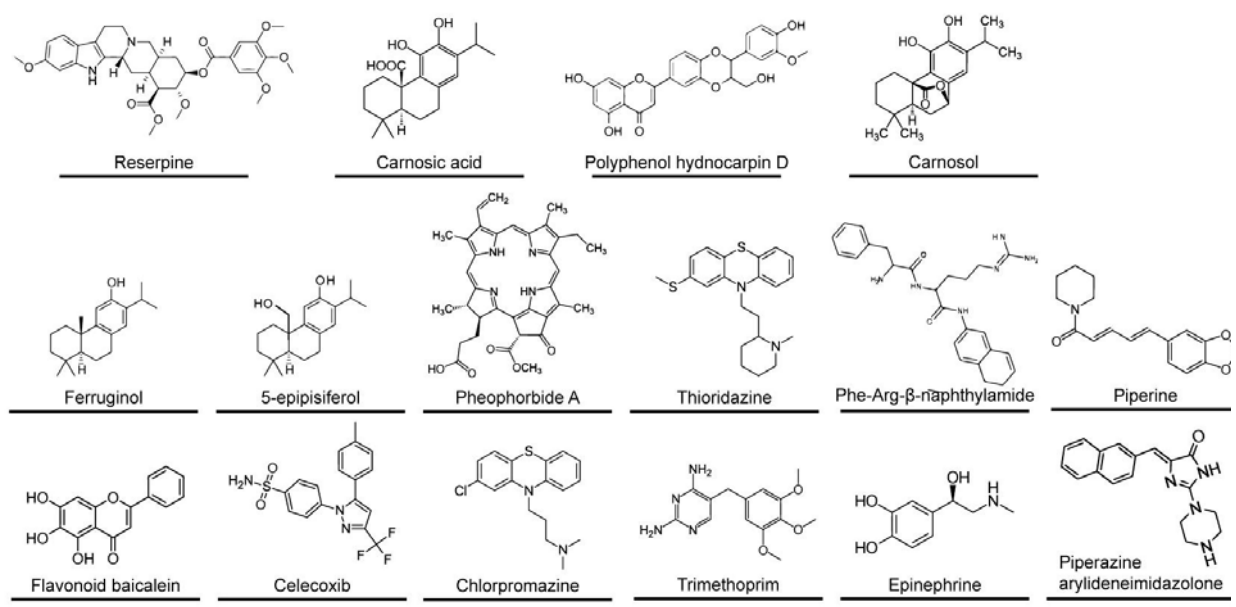


Figure 4: Inhibitors of efflux pumps in Gram-negative bacteria

Celecoxib is a NorA inhibitor that can suppresses drug resistance in the cancer cell with multiple antibiotic classes, including ampicillin, chloramphenicol, kanamycin, and ciprofloxacin (Kalle & Rizvi, 2011). Thioridazine has modest antimicrobial activity and can inhibit both, efflux-mediated and non-mediated resistance mechanisms (Kaatz et al., 2003). MdeA efflux pump is responsible for resistance to several antibiotics, including mupirocin and novobiocins; alkaloid piperine can inhibit MdeA and NorA in *S. aureus*(Jang, 2016).

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 (Pagès & Amaral, 2009). 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 same name, ramA (Bailey et al., 2008; Bohnert et al., 2016). 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 (Bailey et al., 2008; Piddock et al., 2010). Another adjuvant piperazine arylideneimidazolone can inhibit efflux by overexpressing acrAB in *E. coli* and increase susceptibility to clarithromycin, levofloxacin, linezolid, and oxacillin (Bohnert et al., 2016).

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* to novobiocin, fusidic acid and erythromycin (Viljanen & Vaara, 1984). However, due to renal toxicity, PMBN is not used in the clinical sector; it requires developing second-

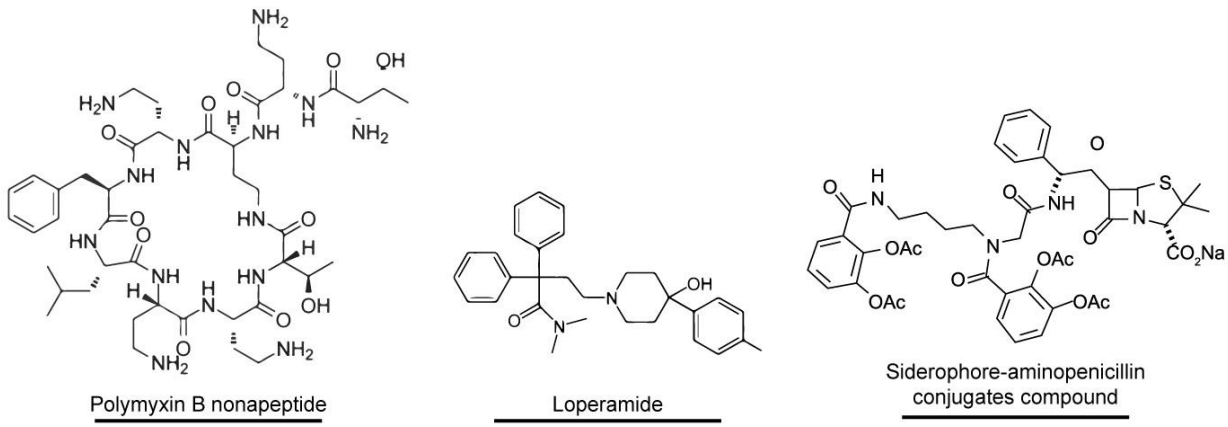


Figure 5: Adjuvants that enhance the uptake of antibiotics

generation analogs with reduced toxicity (Zabawa et al., 2016). Adjuvant loperamide can increase tetracycline uptake in Gram-negative bacteria, including *E. coli, A. baumannii, P. aeruginosa, Salmonella enterica,* and *K. pneumoniae*(Ejim et al., 2011). Pathogenic bacteria use siderophore-specific receptors for iron entry 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*(Möllmann et al., 2009).

Interfering with signaling systems

Interfering with the ability of the bacteria to "switch on" resistance machinery is an alternative method against AMR. 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 (Boudreau et al., 2015).

A prominent signalling 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 (Gotoh et al., 2010; Méjean, 2016). TCS depends on histidine kinase and can control gene expression in response to environmental change by phosphatases and dephosphorylate activity (Gotoh et al., 2010). VraRS system in MRSA is a good example of TCS that allow antibiotic resistance (Belcheva & Golemi-Kotra, 2008). 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

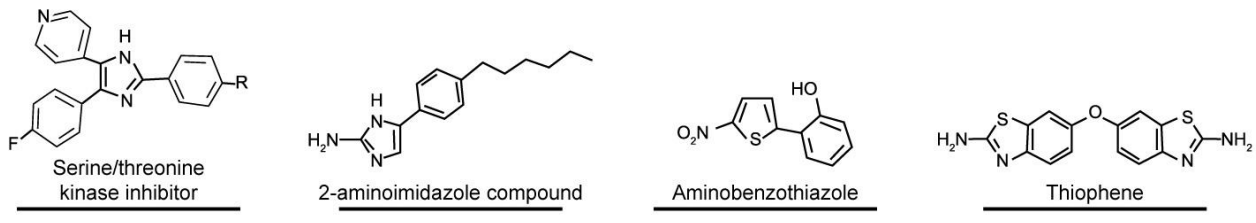


Figure 6: Inhibitors of bacterial signaling systems involved in antibiotic resistance

natural products (Yeagley et al., 2013). Aminobenzothiazole and thiophene (**Figure 6**) exhibited moderate antibiotic activity against *E. coli* and *Bacillus subtilis* by inactivating histidine kinases (Wilke et al., 2015).

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 (Reed et al., 2015). 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 (Wang et al., 2013). 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 (Campbell et al., 2011). However, due to toxicity, tunicamycin cannot be used clinically. Intoxic ticlopidine and benzimidazole tarocin B are used with cefuroxime against wild-type MRSA (Mann et al., 2013).

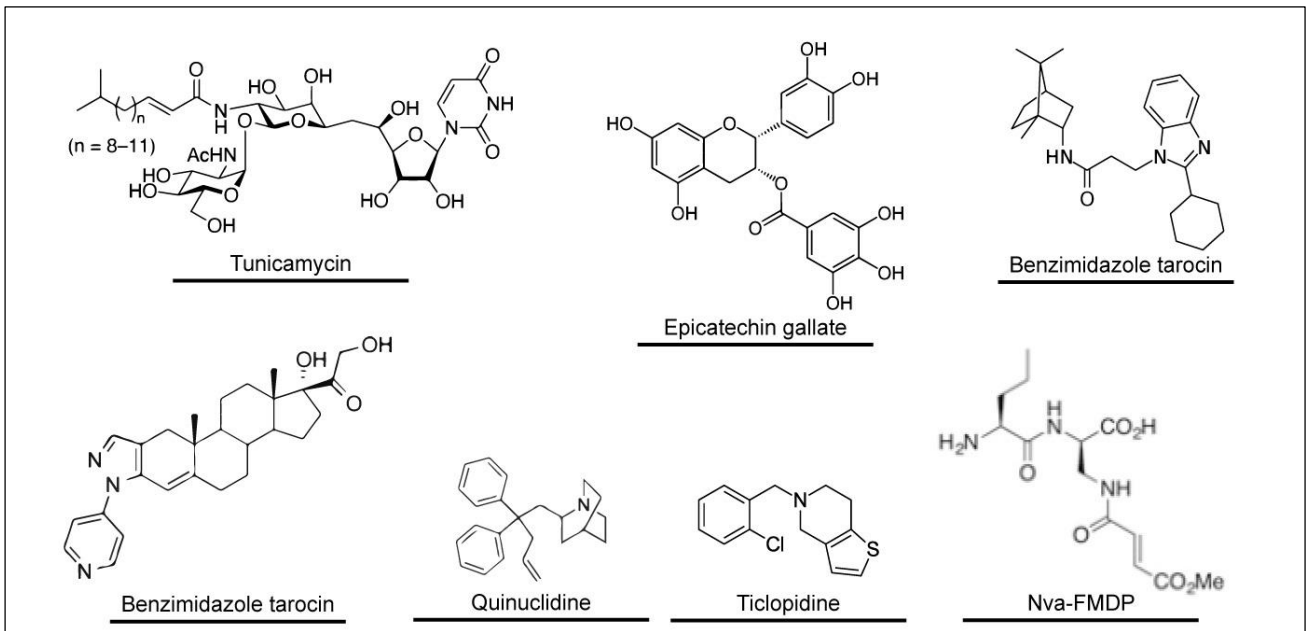


Figure 7: Adjuvants that target cell wall synthesis

The highly conserved cytoskeletal protein FtsZ plays an essential role in cell division (Hurley et al., 2016). Inhibition of FtsZ using thiazolo-pyridine PC190723, enhances the

activity of cell-wall-acting antibiotics at sub-microbicidal concentrations (Tan et al., 2012) Another FtsZ inhibitor is quinuclidine (Chan et al., 2015), used with ceftriaxone against Gram-negative pathogens, including *P. aeruginosa, K. pneumonia,E. coli*, and *A. baumannii* (Nair et al., 2015). Nva-FMDP (**Figure 7**) is an inhibitor of the enzyme encoded by GlmS gene, which is involved in the synthesis of the peptidoglycan precursor (Lee et al., 2011).

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 upregulate neutrophil and downregulate pro-inflammatory cytokines and IFN-c, thus enhance the antibacterial activity of the innate immune system (Mansour et al., 2014). Also, most recently, lactoferritin derivative hLF1-11, displayed antibacterial activity in a rabbit osteomyelitis infection model (Morici et al., 2017). 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 (Guchhait et al., 2015).

New research possibilities

Broad-spectrum antibiotic adjuvants

Broad-spectrum antibiotics have disadvantages, such as triggering hyper-inflammatory responses, disrupting the beneficial microbiome, and developing AMR. Therefore we need to select pathogen-specific antibiotics (Brown & Wright, 2016). 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 AMR 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*(Findlay et al., 2012). Notably, antibiotic-adjuvant conjugates may also encounter pharmacokinetic (PK) problems of their molecular size for tissue uptake and distribution. Recently, tobramycin-based hybrids have been systematically reviewed (Domalaon et al., 2018). However, further study on molecular complexity and intractable chemical synthesis is required to establish the benefit of the hybrids approach.

Conclusion

There is a race between humans and microorganisms for developing new drugs with antibiotic activity versus acquiring resistance mechanisms. The causes of AMR 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 AMR 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 AMR. 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.

Reference

Abreu, A. C., McBain, A. J., & Simoes, M. (2012). Plants as sources of new antimicrobials and resistance-modifying agents. *Natural product reports*, *29*(9), 1007-1021.

Adabi, M., Talebi-Taher, M., Arbabi, L., Afshar, M., Fathizadeh, S., Minaeian, S., Moghadam-Maragheh, N., & Majidpour, A. (2015). Spread of efflux pump overexpressing-mediated fluoroquinolone resistance and multidrug resistance in Pseudomonas aeruginosa by using an efflux pump inhibitor. *Infection & chemotherapy*, *47*(2), 98-104.

Ahmad, M., & Khan, A. U. (2019). Global economic impact of antibiotic resistance: A review. *Journal of global antimicrobial resistance*, *19*, 313-316.

Alcock, B. P., Raphenya, A. R., Lau, T. T., Tsang, K. K., Bouchard, M., Edalatmand, A., Huynh, W., Nguyen, A.-L. V., Cheng, A. A., & Liu, S. (2020). CARD 2020: antibiotic resistome surveillance with the comprehensive antibiotic resistance database. *Nucleic acids research*, *48*(D1), D517-D525.

Allen, H. K., Donato, J., Wang, H. H., Cloud-Hansen, K. A., Davies, J., & Handelsman, J. (2010). Call of the wild: antibiotic resistance genes in natural environments. *Nature reviews microbiology*, *8*(4), 251-259.

America, I. D. S. o. (2011). Combating antimicrobial resistance: policy recommendations to save lives. *Clinical Infectious Diseases*, *52*(suppl_5), S397-S428.

Andersson, D. I., & Hughes, D. (2014). Microbiological effects of sublethal levels of antibiotics. *Nature reviews microbiology*, *12*(7), 465-478.

Aslam, B., Wang, W., Arshad, M. I., Khurshid, M., Muzammil, S., Rasool, M. H., Nisar, M. A., Alvi, R. F., Aslam, M. A., & Qamar, M. U. (2018). Antibiotic resistance: a rundown of a global crisis. *Infection and drug resistance*, *11*, 1645.

Bailey, A. M., Paulsen, I. T., & Piddock, L. J. (2008). RamA confers multidrug resistance in Salmonella enterica via increased expression of acrB, which is inhibited by chlorpromazine. *Antimicrobial agents and chemotherapy*, *52*(10), 3604-3611.

Baker, S. J., Payne, D. J., Rappuoli, R., & De Gregorio, E. (2018). Technologies to address antimicrobial resistance. *Proceedings of the National Academy of Sciences*, *115*(51), 12887-12895.

Ball, P. (2007). The clinical development and launch of amoxicillin/clavulanate for the treatment of a range of community-acquired infections. *International journal of antimicrobial agents*, *30*, 113-117.

Bassetti, M., Ginocchio, F., & Mikulska, M. (2011). New treatment options against gram-negative organisms. *Annual Update in Intensive Care and Emergency Medicine 2011*, 501-515.

Belcheva, A., & Golemi-Kotra, D. (2008). A close-up view of the VraSR two-component system: a mediator of Staphylococcus aureus response to cell wall damage. *Journal of Biological Chemistry*, *283*(18), 12354-12364.

Berendonk, T. U., Manaia, C. M., Merlin, C., Fatta-Kassinos, D., Cytryn, E., Walsh, F., Bürgmann, H., Sørum, H., Norström, M., & Pons, M.-N. (2015). Tackling antibiotic resistance: the environmental framework. *Nature reviews microbiology*, *13*(5), 310-317.

Bhullar, K., Waglechner, N., Pawlowski, A., Koteva, K., Banks, E. D., Johnston, M. D., Barton, H. A., & Wright, G. D. (2012). Antibiotic resistance is prevalent in an isolated cave microbiome. *PloS one*, *7*(4), e34953.

Blair, J., Webber, M. A., Baylay, A. J., Ogbolu, D. O., & Piddock, L. J. (2015). Molecular mechanisms of antibiotic resistance. *Nature reviews microbiology*, *13*(1), 42-51.

Blake, K. L., & O'Neill, A. J. (2013). Transposon library screening for identification of genetic loci participating in intrinsic susceptibility and acquired resistance to antistaphylococcal agents. *Journal of antimicrobial chemotherapy*, *68*(1), 12-16.

Bohnert, J. A., Schuster, S., Kern, W. V., Karcz, T., Olejarz, A., Kaczor, A., Handzlik, J., & Kieć-Kononowicz, K. (2016). Novel piperazine arylideneimidazolones inhibit the AcrAB-TolC pump in Escherichia coli and simultaneously act as fluorescent membrane probes in a combined real-time influx and efflux assay. *Antimicrobial agents and chemotherapy*, *60*(4), 1974-1983.

Boudreau, M. A., Fishovitz, J., Llarrull, L. I., Xiao, Q., & Mobashery, S. (2015). Phosphorylation of BlaR1 in manifestation of antibiotic resistance in methicillin-resistant Staphylococcus aureus and its abrogation by small molecules. *ACS Infectious Diseases*, *1*(10), 454-459.

Brown, E. D., & Wright, G. D. (2016). Antibacterial drug discovery in the resistance era. *Nature*, *529*(7586), 336-343.

Bush, K. (2015a). Investigational agents for the treatment of Gram-negative bacterial infections: a reality check. *ACS Infectious Diseases*, *1*(11), 509-511.

Bush, K. (2015b). A resurgence of β-lactamase inhibitor combinations effective against multidrug-resistant Gram-negative pathogens. *International journal of antimicrobial agents*, *46*(5), 483-493.

Campbell, J., Singh, A. K., Santa Maria Jr, J. P., Kim, Y., Brown, S., Swoboda, J. G., Mylonakis, E., Wilkinson, B. J., & Walker, S. (2011). Synthetic lethal compound combinations reveal a fundamental connection between wall teichoic acid and peptidoglycan biosyntheses in Staphylococcus aureus. *ACS chemical biology*, *6*(1), 106-116.

Chan, F.-Y., Sun, N., Leung, Y.-C., & Wong, K.-Y. (2015). Antimicrobial activity of a quinuclidine-based FtsZ inhibitor and its synergistic potential with β-lactam antibiotics. *The Journal of Antibiotics*, *68*(4), 253-258.

Chiem, K., Fuentes, B. A., Lin, D. L., Tran, T., Jackson, A., Ramirez, M. S., & Tolmasky, M. E. (2015). Inhibition of aminoglycoside 6′-N-acetyltransferase type Ib-mediated amikacin resistance in Klebsiella pneumoniae by zinc and copper pyrithione. *Antimicrobial agents and chemotherapy*, *59*(9), 5851-5853.

D'Costa, V. M., King, C. E., Kalan, L., Morar, M., Sung, W. W., Schwarz, C., Froese, D., Zazula, G., Calmels, F., & Debruyne, R. (2011). Antibiotic resistance is ancient. *Nature*, *477*(7365), 457-461.

Davies, J., & Davies, D. (2010). Origins and evolution of antibiotic resistance. *Microbiology and molecular biology reviews*, *74*(3), 417-433.

Domalaon, R., Idowu, T., Zhanel, G. G., & Schweizer, F. (2018). Antibiotic hybrids: the next generation of agents and adjuvants against Gram-negative pathogens? *Clinical microbiology reviews*, *31*(2), e00077-00017.

Durkin, M. J., Feng, Q., Warren, K., Lockhart, P. B., Thornhill, M. H., Munshi, K. D., Henderson, R. R., Hsueh, K., & Fraser, V. J. (2018). Assessment of inappropriate antibiotic prescribing among a large cohort of general dentists in the United States. *The Journal of the American Dental Association*, *149*(5), 372-381. e371.

Ebmeyer, S., Kristiansson, E., & Larsson, D. (2021). A framework for identifying the recent origins of mobile antibiotic resistance genes. *Communications biology*, *4*(1), 1-10.

Ehmann, D. E., Jahić, H., Ross, P. L., Gu, R.-F., Hu, J., Kern, G., Walkup, G. K., & Fisher, S. L. (2012). Avibactam is a covalent, reversible, non–β-lactam β-lactamase inhibitor. *Proceedings of the National Academy of Sciences*, *109*(29), 11663-11668.

Ejim, L., Farha, M. A., Falconer, S. B., Wildenhain, J., Coombes, B. K., Tyers, M., Brown, E. D., & Wright, G. D. (2011). Combinations of antibiotics and nonantibiotic drugs enhance antimicrobial efficacy. *Nature chemical biology*, *7*(6), 348-350.

Feder, M., Purta, E., Koscinski, L., Čubrilo, S., Maravic Vlahovicek, G., & Bujnicki, J. M. (2008). Virtual screening and experimental verification to identify potential inhibitors of the ErmC methyltransferase responsible for bacterial resistance against macrolide antibiotics. *ChemMedChem: Chemistry Enabling Drug Discovery*, *3*(2), 316-322.

Findlay, B., Zhanel, G. G., & Schweizer, F. (2012). Neomycin–phenolic conjugates: Polycationic amphiphiles with broad-spectrum antibacterial activity, low hemolytic activity and weak serum protein binding. *Bioorganic & medicinal chemistry letters*, *22*(4), 1499-1503.

Gao, F., Yan, X., Shakya, T., Baettig, O. M., Ait-Mohand-Brunet, S., Berghuis, A. M., Wright, G. D., & Auclair, K. (2006). Synthesis and structure− activity relationships of truncated bisubstrate inhibitors of aminoglycoside 6 '-N-acetyltransferases. *Journal of medicinal chemistry*, *49*(17), 5273-5281.

Gill, E. E., Franco, O. L., & Hancock, R. E. (2015). Antibiotic adjuvants: diverse strategies for controlling drug-resistant pathogens. *Chemical biology & drug design*, *85*(1), 56-78.

Girlich, D., Bonnin, R. A., Dortet, L., & Naas, T. (2020). Genetics of acquired antibiotic resistance genes in Proteus spp. *Frontiers in microbiology*, *11*, 256.

González-Bello, C. (2017). Antibiotic adjuvants–A strategy to unlock bacterial resistance to antibiotics. *Bioorganic & medicinal chemistry letters*, *27*(18), 4221-4228.

Gotoh, Y., Eguchi, Y., Watanabe, T., Okamoto, S., Doi, A., & Utsumi, R. (2010). Two-component signal transduction as potential drug targets in pathogenic bacteria. *Current opinion in microbiology*, *13*(2), 232-239.

Griffith, D. C., Loutit, J. S., Morgan, E. E., Durso, S., & Dudley, M. N. (2016). Phase 1 study of the safety, tolerability, and pharmacokinetics of the β-lactamase inhibitor vaborbactam (RPX7009) in healthy adult subjects. *Antimicrobial agents and chemotherapy*, *60*(10), 6326-6332.

Guchhait, G., Altieri, A., Gorityala, B., Yang, X., Findlay, B., Zhanel, G. G., Mookherjee, N., & Schweizer, F. (2015). Amphiphilic tobramycins with immunomodulatory properties. *Angewandte Chemie International Edition*, *54*(21), 6278-6282.

Gutierrez-Lugo, M.-T., Baker, H., Shiloach, J., Boshoff, H., & Bewley, C. A. (2009). Dequalinium, a new inhibitor of Mycobacterium tuberculosis mycothiol ligase identified by high-throughput screening. *SLAS Discovery*, *14*(6), 643-652.

He, Y., Yuan, Q., Mathieu, J., Stadler, L., Senehi, N., Sun, R., & Alvarez, P. J. (2020). Antibiotic resistance genes from livestock waste: Occurrence, dissemination, and treatment. *NPJ Clean Water*, *3*(1), 1-11.

Hernando-Amado, S., Coque, T. M., Baquero, F., & Martínez, J. L. (2019). Defining and combating antibiotic resistance from One Health and Global Health perspectives. *Nature microbiology*, *4*(9), 1432-1442.

Hernick, M. (2013). Mycothiol: a target for potentiation of rifampin and other antibiotics against Mycobacterium tuberculosis. *Expert review of anti-infective therapy*, *11*(1), 49-67.

Hinchliffe, P., González, M. M., Mojica, M. F., González, J. M., Castillo, V., Saiz, C., Kosmopoulou, M., Tooke, C. L., Llarrull, L. I., & Mahler, G. (2016). Cross-class metallo-β-lactamase inhibition by bisthiazolidines reveals multiple binding modes. *Proceedings of the National Academy of Sciences*, *113*(26), E3745-E3754.

Hoffman, S. J., Caleo, G. M., Daulaire, N., Elbe, S., Matsoso, P., Mossialos, E., Rizvi, Z., & Røttingen, J.-A. (2015). Strategies for achieving global collective action on antimicrobial resistance. *Bulletin of the World Health Organization*, *93*, 867-876.

Holmes, A. H., Moore, L. S., Sundsfjord, A., Steinbakk, M., Regmi, S., Karkey, A., Guerin, P. J., & Piddock, L. J. (2016). Understanding the mechanisms and drivers of antimicrobial resistance. *The Lancet*, *387*(10014), 176-187.

Hurley, K. A., Santos, T. M., Nepomuceno, G. M., Huynh, V., Shaw, J. T., & Weibel, D. B. (2016). Targeting the bacterial division protein FtsZ. *Journal of medicinal chemistry*, *59*(15), 6975-6998.

Jang, S. (2016). Multidrug efflux pumps in Staphylococcus aureus and their clinical implications. *Journal of Microbiology*, *54*(1), 1-8.

Jovetic, S., Zhu, Y., Marcone, G. L., Marinelli, F., & Tramper, J. (2010). β-Lactam and glycopeptide antibiotics: first and last line of defense? *Trends in biotechnology*, *28*(12), 596-604.

Kaatz, G. W., Moudgal, V. V., Seo, S. M., & Kristiansen, J. E. (2003). Phenothiazines and thioxanthenes inhibit multidrug efflux pump activity in Staphylococcus aureus. *Antimicrobial agents and chemotherapy*, *47*(2), 719-726.

Kalle, A. M., & Rizvi, A. (2011). Inhibition of bacterial multidrug resistance by celecoxib, a cyclooxygenase-2 inhibitor. *Antimicrobial agents and chemotherapy*, *55*(1), 439-442.

King, A. M., Reid-Yu, S. A., Wang, W., King, D. T., De Pascale, G., Strynadka, N. C., Walsh, T. R., Coombes, B. K., & Wright, G. D. (2014). Aspergillomarasmine A overcomes metallo-β-lactamase antibiotic resistance. *Nature*, *510*(7506), 503-506.

Klein, E. Y., Milkowska-Shibata, M., Tseng, K. K., Sharland, M., Gandra, S., Pulcini, C., & Laxminarayan, R. (2021). Assessment of WHO antibiotic consumption and access targets in 76 countries, 2000–15: an analysis of pharmaceutical sales data. *The Lancet Infectious Diseases*, *21*(1), 107-115.

Ko¨ hler, T., Michéa-Hamzehpour, M., Henze, U., Gotoh, N., Kocjancic Curty, L., & Pechère, J. C. (1997). Characterization of MexE–MexF–OprN, a positively regulated multidrug efflux system of Pseudomonas aeruginosa. *Molecular microbiology*, *23*(2), 345-354.

Kostyanev, T., Bonten, M., O'brien, S., Steel, H., Ross, S., François, B., Tacconelli, E., Winterhalter, M., Stavenger, R., & Karlén, A. (2016). The Innovative Medicines Initiative's New Drugs for Bad Bugs programme: European public–private partnerships for the development of new strategies to tackle antibiotic resistance. *Journal of antimicrobial chemotherapy*, *71*(2), 290-295.

Labby, K. J., & Garneau-Tsodikova, S. (2013). Strategies to overcome the action of aminoglycoside-modifying enzymes for treating resistant bacterial infections. *Future medicinal chemistry*, *5*(11), 1285-1309.

Landers, T., & Kavanagh, K. T. (2016). Is the Presidential Advisory Council on Combating Antibiotic Resistance missing opportunities? *American Journal of Infection Control*, *44*(11), 1356-1359.

Lapuebla, A., Abdallah, M., Olafisoye, O., Cortes, C., Urban, C., Quale, J., & Landman, D. (2015). Activity of meropenem combined with RPX7009, a novel β-lactamase inhibitor, against Gram-negative clinical isolates in New York City. *Antimicrobial agents and chemotherapy*, *59*(8), 4856-4860.

Lee, S. H., Jarantow, L. W., Wang, H., Sillaots, S., Cheng, H., Meredith, T. C., Thompson, J., & Roemer, T. (2011). Antagonism of chemical genetic interaction networks resensitize MRSA to β-lactam antibiotics. *Chemistry & biology*, *18*(11), 1379-1389.

Lesho, E. P., & Laguio-Vila, M. (2019). The slow-motion catastrophe of antimicrobial resistance and practical interventions for all prescribers. Mayo Clinic Proceedings,
Lewis, D. (2013). The role of core groups in the emergence and dissemination of antimicrobial-resistant N gonorrhoeae. *Sexually transmitted infections*, *89*(Suppl 4), iv47-iv51.
Li, X.-Z., Plésiat, P., & Nikaido, H. (2015). The challenge of efflux-mediated antibiotic resistance in Gram-negative bacteria. *Clinical microbiology reviews*, *28*(2), 337-418.
Li, Y., Green, K. D., Johnson, B. R., & Garneau-Tsodikova, S. (2015). Inhibition of aminoglycoside acetyltransferase resistance enzymes by metal salts. *Antimicrobial agents and chemotherapy*, *59*(7), 4148-4156.
Lin, D. L., Tran, T., Alam, J. Y., Herron, S. R., Ramirez, M. S., & Tolmasky, M. E. (2014). Inhibition of aminoglycoside 6′-N-acetyltransferase type Ib by zinc: reversal of amikacin resistance in Acinetobacter baumannii and Escherichia coli by a zinc ionophore. *Antimicrobial agents and chemotherapy*, *58*(7), 4238-4241.
Livermore, D. M., & Mushtaq, S. (2013). Activity of biapenem (RPX2003) combined with the boronate β-lactamase inhibitor RPX7009 against carbapenem-resistant Enterobacteriaceae. *Journal of antimicrobial chemotherapy*, *68*(8), 1825-1831.
Lynch III, J. P., Clark, N. M., & Zhanel, G. G. (2013). Evolution of antimicrobial resistance among Enterobacteriaceae (focus on extended spectrum β-lactamases and carbapenemases). *Expert opinion on pharmacotherapy*, *14*(2), 199-210.
MacLean, R. C., & San Millan, A. (2019). The evolution of antibiotic resistance. *Science*, *365*(6458), 1082-1083.
Mann, P. A., Mul er, A., Xiao, L., Pereira, P. M., Yang, C., Ho Lee, S., Wang, H., Trzeciak, J., Schneeweis, J., & Dos Santos, M. M. (2013). Murgocil is a highly bioactive staphylococcal-specific inhibitor of the peptidoglycan glycosyltransferase enzyme MurG. *ACS chemical biology*, *8*(11), 2442-2451.
Mansour, S. C., Pena, O. M., & Hancock, R. E. (2014). Host defense peptides: front-line immunomodulators. *Trends in immunology*, *35*(9), 443-450.
Massip, C., Descours, G., Ginevra, C., Doublet, P., Jarraud, S., & Gilbert, C. (2017). Macrolide resistance in Legionella pneumophila: the role of LpeAB efflux pump. *Journal of antimicrobial chemotherapy*, *72*(5), 1327-1333.
McGarvey, K. M., Queitsch, K., & Fields, S. (2012). Wide variation in antibiotic resistance proteins identified by functional metagenomic screening of a soil DNA library. *Applied and environmental microbiology*, *78*(6), 1708-1714.
McInnes, R. S., McCallum, G. E., Lamberte, L. E., & van Schaik, W. (2020). Horizontal transfer of antibiotic resistance genes in the human gut microbiome. *Current opinion in microbiology*, *53*, 35-43.
Méjean, V. (2016). Two-component regulatory systems: the moment of truth. In (Vol. 167, pp. 1-3).
Melander, R. J., & Melander, C. (2017). Antibiotic adjuvants. *Antibacterials*, 89-118.
Möllmann, U., Heinisch, L., Bauernfeind, A., Köhler, T., & Ankel-Fuchs, D. (2009). Siderophores as drug delivery agents: application of the “Trojan Horse” strategy. *Biometals*, *22*(4), 615-624.
Morici, P., Florio, W., Rizzato, C., Ghelardi, E., Tavanti, A., Rossolini, G., & Lupetti, A. (2017). Synergistic activity of synthetic N-terminal peptide of human lactoferrin in combination with various antibiotics against carbapenem-resistant Klebsiella pneumoniae strains. *European Journal of Clinical Microbiology & Infectious Diseases*, *36*(10), 1739-1748.

Munita, J. M., & Arias, C. A. (2016). Mechanisms of antibiotic resistance. *Microbiology spectrum*, *4*(2), 4.2. 15.

Nair, D. R., Monteiro, J. M., Memmi, G., Thanassi, J., Pucci, M., Schwartzman, J., Pinho, M. G., & Cheung, A. L. (2015). Characterization of a novel small molecule that potentiates β-lactam activity against Gram-positive and Gram-negative pathogens. *Antimicrobial agents and chemotherapy*, *59*(4), 1876-1885.

Nielsen, T. K., Browne, P. D., & Hansen, L. H. (2022). Antibiotic resistance genes are differentially mobilized according to resistance mechanism. *GigaScience*, *11*.

Page, M. G., Dantier, C., Desarbre, E., Gaucher, B., Gebhardt, K., & Schmitt-Hoffmann, A. (2011). In vitro and in vivo properties of BAL30376, a β-lactam and dual β-lactamase inhibitor combination with enhanced activity against Gram-negative bacilli that express multiple β-lactamases. *Antimicrobial agents and chemotherapy*, *55*(4), 1510-1519.

Pagès, J.-M., & Amaral, L. (2009). Mechanisms of drug efflux and strategies to combat them: challenging the efflux pump of Gram-negative bacteria. *Biochimica et Biophysica Acta (BBA)-Proteins and Proteomics*, *1794*(5), 826-833.

Pan, Y.-p., Xu, Y.-h., Wang, Z.-x., Fang, Y.-p., & Shen, J.-l. (2016). Overexpression of MexAB-OprM efflux pump in carbapenem-resistant Pseudomonas aeruginosa. *Archives of microbiology*, *198*(6), 565-571.

Papp-Wallace, K. M., & Bonomo, R. A. (2016). New β-lactamase inhibitors in the clinic. *Infectious Disease Clinics*, *30*(2), 441-464.

Paukner, S., Hesse, L., Prezelj, A., S̆o majer, T., & Urleb, U. (2009). In vitro activity of LK-157, a novel tricyclic carbapenem as broad-spectrum β-lactamase inhibitor. *Antimicrobial agents and chemotherapy*, *53*(2), 505-511.

Payne, D. J., Miller, L. F., Findlay, D., Anderson, J., & Marks, L. (2015). Time for a change: addressing R&D and commercialization challenges for antibacterials. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *370*(1670), 20140086.

Pazda, M., Kumirska, J., Stepnowski, P., & Mulkiewicz, E. (2019). Antibiotic resistance genes identified in wastewater treatment plant systems–a review. *Science of the Total Environment*, *697*, 134023.

Piddock, L. J., Garvey, M. I., Rahman, M. M., & Gibbons, S. (2010). Natural and synthetic compounds such as trimethoprim behave as inhibitors of efflux in Gram-negative bacteria. *Journal of antimicrobial chemotherapy*, *65*(6), 1215-1223.

Pieren, M., & Tigges, M. (2012). Adjuvant strategies for potentiation of antibiotics to overcome antimicrobial resistance. *Current opinion in pharmacology*, *12*(5), 551-555.

Pokharel, S., Shrestha, P., & Adhikari, B. (2020). Antimicrobial use in food animals and human health: time to implement 'One Health'approach. *Antimicrobial Resistance & Infection Control*, *9*(1), 1-5.

Poole, K. (2007). Efflux pumps as antimicrobial resistance mechanisms. *Annals of medicine*, *39*(3), 162-176.

Putman, M., van Veen, H. W., & Konings, W. N. (2000). Molecular properties of bacterial multidrug transporters. *Microbiology and molecular biology reviews*, *64*(4), 672-693.

Rahman, S., Sarker, M. S., Aralaguppe, S. G., Sarwar, G., Khan, S. I., & Rahman, M. (2022). Drug resistance pattern among ART-naive clients attending an HIV testing and counseling center in Dhaka, Bangladesh. *Journal of Medical Virology*, *94*(2), 787-790.

Ramirez, M. S., & Tolmasky, M. E. (2010). Aminoglycoside modifying enzymes. *Drug resistance updates*, *13*(6), 151-171.

Ramón-García, S., Ng, C., Anderson, H., Chao, J. D., Zheng, X., Pfeifer, T., Av-Gay, Y., Roberge, M., & Thompson, C. J. (2011). Synergistic drug combinations for tuberculosis therapy identified by a novel high-throughput screen. *Antimicrobial agents and chemotherapy*, *55*(8), 3861-3869.

Randall, C. P., Mariner, K. R., Chopra, I., & O'Neill, A. J. (2013). The target of daptomycin is absent from Escherichia coli and other gram-negative pathogens. *Antimicrobial agents and chemotherapy*, *57*(1), 637-639.

Reed, P., Atilano, M. L., Alves, R., Hoiczyk, E., Sher, X., Reichmann, N. T., Pereira, P. M., Roemer, T., Filipe, S. R., & Pereira-Leal, J. B. (2015). Staphylococcus aureus survives with a minimal peptidoglycan synthesis machine but sacrifices virulence and antibiotic resistance. *Plos pathogens*, *11*(5), e1004891.

Roemer, T., & Boone, C. (2013). Systems-level antimicrobial drug and drug synergy discovery. *Nature chemical biology*, *9*(4), 222-231.

Schmidt, J., Kunderova, M., Pilbauerova, N., & Kapitan, M. (2021). A Review of Evidence-Based Recommendations for Pericoronitis Management and a Systematic Review of Antibiotic Prescribing for Pericoronitis among Dentists: Inappropriate Pericoronitis Treatment Is a Critical Factor of Antibiotic Overuse in Dentistry. *International Journal of environmental research and public health*, *18*(13), 6796.

Schmitz, F., Hertel, B., Hofmann, B., Scheuring, S., Verhoef, J., Fluit, A., Heinz, H., Köhrer, K., & Jones, M. (1998). Relationship between mutations in the coding and promoter regions of the norA genes in 42 unrelated clinical isolates of Staphylococcus aureus and the MICs of norfloxacin for these strains. *The Journal of antimicrobial chemotherapy*, *42*(4), 561-563.

Shakya, T., Stogios, P. J., Waglechner, N., Evdokimova, E., Ejim, L., Blanchard, J. E., McArthur, A. G., Savchenko, A., & Wright, G. D. (2011). A small molecule discrimination map of the antibiotic resistance kinome. *Chemistry & biology*, *18*(12), 1591-1601.

Sharma, N., Chhillar, A. K., Dahiya, S., Choudhary, P., Punia, A., & Gulia, P. (2021). Antibiotic adjuvants: A promising approach to combat multidrug resistant bacteria. *Current Drug Targets*, *22*(12), 1334-1345.

Shlaes, D. M. (2013). New β-lactam–β-lactamase inhibitor combinations in clinical development. *Annals of the new York Academy of Sciences*, *1277*(1), 105-114.

Spellberg, B., Srinivasan, A., & Chambers, H. F. (2016). New societal approaches to empowering antibiotic stewardship. *Jama*, *315*(12), 1229-1230.

Suga, T., Ishii, T., Iwatsuki, M., Yamamoto, T., Nonaka, K., Masuma, R., Matsui, H., Hanaki, H., Ōmura, S., & Shiomi, K. (2012). Aranorosin circumvents arbekacin-resistance in MRSA by inhibiting the bifunctional enzyme AAC (6′)/APH (2 ″). *The Journal of Antibiotics*, *65*(10), 527-529.

Talebi Bezmin Abadi, A., Rizvanov, A. A., Haertlé, T., & Blatt, N. L. (2019). World Health Organization report: current crisis of antibiotic resistance. *BioNanoScience*, *9*(4), 778-788.

Tan, C. M., Therien, A. G., Lu, J., Lee, S. H., Caron, A., Gill, C. J., Lebeau-Jacob, C., Benton-Perdomo, L., Monteiro, J. M., & Pereira, P. M. (2012). Restoring methicillin-resistant Staphylococcus aureus susceptibility to β-lactam antibiotics. *Science translational medicine*, *4*(126), 126ra135-126ra135.

Tiseo, K., Huber, L., Gilbert, M., Robinson, T. P., & Van Boeckel, T. P. (2020). Global trends in antimicrobial use in food animals from 2017 to 2030. *Antibiotics*, *9*(12), 918.

Umscheid, C. A., Mitchell, M. D., Doshi, J. A., Agarwal, R., Williams, K., & Brennan, P. J. (2011). Estimating the proportion of healthcare-associated infections that are reasonably preventable and the related mortality and costs. *Infection Control & Hospital Epidemiology*, *32*(2), 101-114.

Ventola, C. L. (2015). The antibiotic resistance crisis: part 1: causes and threats. *Pharmacy and therapeutics*, *40*(4), 277.

Viljanen, P., & Vaara, M. (1984). Susceptibility of gram-negative bacteria to polymyxin B nonapeptide. *Antimicrobial agents and chemotherapy*, *25*(6), 701-705.

Volkers, G., Palm, G. J., Weiss, M. S., Wright, G. D., & Hinrichs, W. (2011). Structural basis for a new tetracycline resistance mechanism relying on the TetX monooxygenase. *FEBS letters*, *585*(7), 1061-1066.

Waksman, S. A. (1947). What is an antibiotic or an antibiotic substance? *Mycologia*, *39*(5), 565-569.

Walsh, C. (2000). Molecular mechanisms that confer antibacterial drug resistance. *Nature*, *406*(6797), 775-781.

Wang, H., Gill, C. J., Lee, S. H., Mann, P., Zuck, P., Meredith, T. C., Murgolo, N., She, X., Kales, S., & Liang, L. (2013). Discovery of wall teichoic acid inhibitors as potential anti-MRSA β-lactam combination agents. *Chemistry & biology*, *20*(2), 272-284.

Wellington, E. M., Boxall, A. B., Cross, P., Feil, E. J., Gaze, W. H., Hawkey, P. M., Johnson-Rollings, A. S., Jones, D. L., Lee, N. M., & Otten, W. (2013). The role of the natural environment in the emergence of antibiotic resistance in Gram-negative bacteria. *The Lancet Infectious Diseases*, *13*(2), 155-165.

Wilke, K. E., Francis, S., & Carlson, E. E. (2015). Inactivation of multiple bacterial histidine kinases by targeting the ATP-binding domain. *ACS chemical biology*, *10*(1), 328-335.

Wright, G. D. (2005). Bacterial resistance to antibiotics: enzymatic degradation and modification. *Advanced drug delivery reviews*, *57*(10), 1451-1470.

Wright, G. D. (2014). Something old, something new: revisiting natural products in antibiotic drug discovery. *Canadian journal of microbiology*, *60*(3), 147-154.

Yeagley, A. A., Su, Z., McCullough, K. D., Worthington, R. J., & Melander, C. (2013). N-substituted 2-aminoimidazole inhibitors of MRSA biofilm formation accessed through direct 1, 3-bis (tert-butoxycarbonyl) guanidine cyclization. *Organic & biomolecular chemistry*, *11*(1), 130-137.

Zabawa, T. P., Pucci, M. J., Parr Jr, T. R., & Lister, T. (2016). Treatment of Gram-negative bacterial infections by potentiation of antibiotics. *Current opinion in microbiology*, *33*, 7-12.

Zhu, L., Lin, J., Ma, J., Cronan, J. E., & Wang, H. (2010). Triclosan resistance of Pseudomonas aeruginosa PAO1 is due to FabV, a triclosan-resistant enoyl-acyl carrier protein reductase. *Antimicrobial agents and chemotherapy*, *54*(2), 689-698.

Zimlichman, E., Henderson, D., Tamir, O., Franz, C., Song, P., Yamin, C. K., Keohane, C., Denham, C. R., & Bates, D. W. (2013). Health care–associated infections: a meta-analysis of costs and financial impact on the US health care system. *JAMA internal medicine*, *173*(22), 2039-2046.