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Tackling Microbial Resistance and Emerging Pathogens with Next-Generation Antibiotics

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Abstract: In the 19th century, the discovery of penicillin revolutionized medicine, saving millions from infectious diseases. People believed that they had won the war against infections. However, the misuse and abuse of antimicrobial agents are accompanied by major ramifications like antimicrobial resistance, creating drug-resistant superbugs. This issue is

concerning worldwide, as dwindling effective antibiotics lead to rising healthcare costs, re-hospitalization, and disease severity. Consequently, multiple initiatives have been undertaken to address these phenomena, including the development of antimicrobials with novel modes of action. Without novel discoveries of newer antimicrobial agents, we may face the risk of entering a post-antibiotic era where uncomplicated infections become untreatable. Ultimately, the morbidity and mortality rate would rise higher than in the pre-antibiotic era. This study highlights the recent developments in antimicrobials over the past five years and explores the strategies employed by the new generation of drugs to act against resistance. For example, we discuss the treatment of Carbapenem-resistant *Enterobacteriaceae*, such as *Klebsiella pneumoniae* Carbapenamase-producing Gram-negative bacteria, by using meropenem-vaborbactam. Plazomicin, lacking a hydroxyl group, effectively combats metallo-beta-lactamase, which meropenem-vaborbactam is unable to address. It is also preferred over tobramycin and gentamicin due to its hydroxyethyl group. Furthermore, we explore the conjugation of nanoparticles with antibiotics, which demonstrated synergistic effects and positive outcomes on different bacterial resistance. Mechanisms include increased drug adhesion to bacterial cell walls, generating oxidative stress, and causing mistranslation by detaching ribosomes from tRNA. Additionally, the IspH inhibitors like 4'-flurouridine targeting the MEP pathway which is also included in the discussion. This report thoroughly examines newer generations and classes of antibiotics, highlighting the improvements made by scientists to combat bacterial resistance effectively.

Keywords: Meropenem-vaborbactam against KPC; plazomicin towards CRE; nanoparticle conjugation with antibiotic; ceftaroline; MRSA superbug; DAIA; MEP pathway; SDG 3 Good health and well-being

1. Introduction

Until the late 19th century, aseptic measures were not commonly enforced despite the availability of sterilized surgical equipment. Surgeons initially resisted these precautions, leading to a high surgical infection risk. Morbidity and mortality rates at the time skyrocketed at an alarming rate, and patients were more likely to die after surgery than the soldiers battling at the Waterloo frontline[1]. By the early 20th century, surgical asepsis was more commonly accepted and practised, significantly reducing post-surgical infections, though other infection sources persisted. In 1928, Alexander Fleming discovered penicillin G (penG), revolutionising medicine by effectively targeting Gram-positive cocci. Despite its success, improper use quickly developed antimicrobial resistance, exemplified by penicillin-resistant

Staphylococcus aureus in 1942. Therefore, this phenomenon highlights the need for responsible antibiotic use[2–4].

Antimicrobial drugs are the cornerstone of modern medicine. However, the emergence and spread of antimicrobial resistance (AMR) impact the efficacy of standard therapy, increasing the risk of disease spread, severe illnesses, disability, and death. AMR is known as the ability of pathogens to resist the drugs designed to inhibit their pathogenicity. It is a natural process that occurs over time through genetic changes in pathogens, which may be accelerated by multiple drivers, especially the misuse and overuse of antimicrobials in humans, animals, and plants[5].

AMR poses the most critical challenge in contemporary healthcare, profoundly impacting clinical settings worldwide. The excessive dependence on antibiotics has resulted in the emergence of antibiotic-resistant bacteria known as superbugs, which complicates the clinical management of patients. When the microorganisms resist the standard antimicrobial treatment, previously treatable infections become progressively intractable. The loss of effective first-line antimicrobials, in turn, causes reliance on second and third-line therapies like polymyxins and carbapenems. These last resorts often expose patients to high toxicity risks and a greater financial burden[6,7]. For instance, infections caused by Methicillin-resistant *S. aureus* (MRSA) necessitate alternative therapies such as vancomycin. This antibiotic can lead to a series of complications that require therapeutic drug monitoring to protect patients from developing severe toxicities caused by vancomycin, including organ failure and prolonged recovery. However, *S. aureus* isolates with resistance to vancomycin have also emerged in recent years[8]. These findings highlighted a clear trend: the more antibiotics were used, the more resistance emerged, leading to a constant need for new antimicrobials[6]. This scenario further complicates the clinical setting, which is detrimental to patient care and healthcare burden.

Additionally, patients infected with multidrug-resistant (MDR) pathogens usually experience extended hospitalisation due to limited treatment options available, leading to a slower recovery time. This prolongation not only affects patient care but also drains the healthcare system resources as the healthcare facilities, diagnostic equipment, and intensive care are overcrowded, thus negatively impacting economic productivity [6]. As reported by Nelson *et al*.[9] in a collaborative study with the Centers for Disease Control and Prevention (CDC), the authors found that the national costs associated with the six notable MDR infections can be substantial at more than USD 4.6 billion annually for hospital- and community-onset infections. This tremendous amount is attributed to the management of AMR extending to infection control measures like isolation procedures, patient monitoring, antimicrobial stewardship programs, and enhanced hygiene adherence. According to World

Bank estimates, AMR could result in an additional USD 1 trillion in healthcare expenses by 2050 and up to USD 3.4 trillion in gross domestic product (GDP) losses annually by 2030[10]. This thread suggests that underprivileged communities will eventually be impacted, and the poverty rate will continue to surge. Thus, the disastrous amount of healthcare expenses, expensive and intensive treatments, and resource utilisation escalation have direct monetary effects on healthcare.

The most alarming impact is the sharp rise in morbidity and mortality rates. Every year, around 2.8 million antibiotic-resistant illnesses and 35,000 deaths due to AMR happen in the United States, according to the CDC's 2019 Antibiotic Resistance Threats Report[11]. AMR suppresses the immune system's capacity to fight infectious diseases, leading to health complications in patients with weak immune systems or chronic conditions. Furthermore, AMR burdens the healthcare system through secondary effects by jeopardising common surgical interventions. The high AMR prevalence prevents the use of antimicrobials that are essential to decrease infection risk during surgical procedures and post-operative care[7]. Also, AMR increases the failure rate of organ transplants and chemotherapy. Patients undergoing chemotherapy are found to have impaired immune systems and high susceptibility to infections, thus preventing physicians from antibiotics administration. Hence, it is crucial to optimise the use of current antimicrobials, and the discovery of novel antimicrobials is critically essential to protect immune-suppressed patients[12].

To date, researchers have mainly focused on developing newer generations of antimicrobial agents to combat known antibiotic resistance, such as MRSA, which is commonly known as superbug, as well as to treat emerging pathogens such as EBOLA or SARS-CoV-2. This article mainly focuses on novel antibacterial agents, including meropenem-vaborbactam, plazomicin, nanoparticle conjugation with antibiotics, and several newer generations and classes of antibiotics (Figure 1). The challenges in addressing antimicrobial resistance, emerging pathogens that lead to resistance, and the resistance mechanisms were also explored.

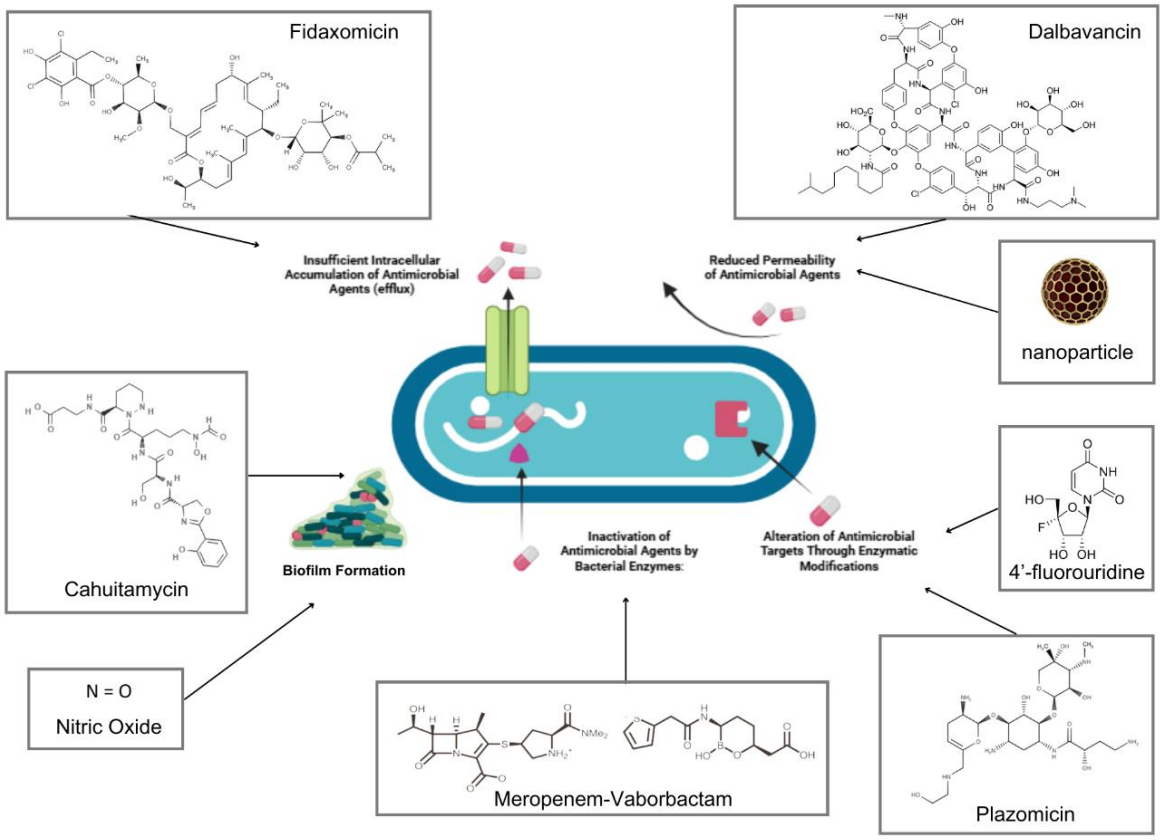

Figure 1. The mechanism of resistance of common antibiotics and the molecular structure of next-generation antimicrobials (NGAs). The resistance mechanisms include insufficient intracellular accumulation of antimicrobial agents by efflux, reduced permeability of antimicrobial agents, alteration of antimicrobial targets through enzymatic modifications, and biofilm formation. Fidaxomicin emerges as an NGA that tackles the efflux system while antibiotic-nanoparticles conjugation and dalbavancin target the permeability of cell membranes. Furthermore, Plazomicin and DAIAs like 2'-fluorouridine target the N-acetyltransferase AAC (6') and IsPH enzymes. The addition of vaborbactam enhances meropenem's activity against beta-lactamase-producing microorganisms. In addition, c-di-GMP receptor inhibitors like cahuitamycin and nitric oxide disrupt biofilm formation and bacterial motility.

2. Emerging Pathogens that Develop Antimicrobial Resistance

2.1. Acinetobacter baumannii

Acinetobacter baumannii, Gram-negative and aerobic coccobacilli, has developed extensive AMR, increasing the mortality rate in intensive care units. *A. baumannii* is classified by the World Health Organization (WHO) as part of the "ESKAPE" group. This pathogen evades the bactericidal effect of antibiotics' bactericidal activity, subsequently developing resistant strains[13]. Also, as the pathogen may adapt to dry conditions for a long time, it persists in the hospital environment as a biofilm. Biofilm formation is the primary

resistance mechanism of *A. baumanii*, as biofilms limit the diffusion of antimicrobials to the site of action[14]. This opportunistic pathogen thus results in hospital-acquired infections worldwide, particularly in immunocompromised patients with central venous catheters and nosocomial infections. Its resistant strains often increase the in-hospital length of stay and mortality. *A. baumannii* infections, ranging from ventilator-associated pneumonia, bloodstream infections, urinary tract infections, skin wound infections, and meningitis, are associated with previous antibiotics and medical devices[15].

In the 1970s, *A. baumannii* were susceptible to common carbapenems like imipenem and meropenem, which show significant bactericidal effects. However, with the emergence of resistant strains, these agents were replaced by minocycline/tigecycline, although significant rates have been recorded[16]. Also, colistin/tigecycline is reported as a last resort for treating resistant strains of *A. baumannii* infections; however, colistin-resistant strains have also been reported[17]. *A. baumannii* develops resistance against multiple mechanisms, including expression of beta-lactamase enzyme, upregulation of efflux pumps (MFS, MATE, RND, SMR), alteration of the antimicrobial target at penicillin-binding protein, reduced permeability of the outer membrane, and enzymatic modification of antibiotics[18]. Hence, such an alarming AMR has anticipated the need to discover novel antimicrobials.

2.2. Mycobacterium abscessus Complex

Mycobacterium abscessus complex (MABC) is a significant mycobacterial isolate associated with pulmonary infections like cystic fibrosis[19]. MABC comprises three sub-species: *M. abscessus* subsp. *abscessus* (Mab), *M. abscessus* subsp. *Bolletii* and *M. abscessus* subsp. *massiliense* (*M. massiliense*)[20].

The current treatment regimens for MABC include multidrug therapy with macrolide and aminoglycoside or beta-lactams. However, recent studies have reported MABC strains with genetic polymorphisms of target genes that lead to resistance against the above antibiotics[21–23]. For instance, the mutations in the aminoglycoside-modifying enzymes lead to macrolide resistance[23]; the truncated *erm* (41) gene in Mab leads to different degrees of macrolide resistance in different subspecies[22]. For instance, the point mutations in the *erm* gene develop resistance to clarithromycin[23].

Moreover, MABC is naturally resistant to many antibiotics, such as rifamycin, tetracyclines, and beta-lactam. Gorzynski *et al*.[24] have reported that such resistance is attributed to the 16 mutants on the surface transport systems, such as efflux pumps, porins, and carrier membrane enzymes. Furthermore, the lipophilic cell walls of mycobacterium

serve as substantial physical barriers against hydrophilic antibiotics while beta-lactamase inactivates the antibiotic structures. Given the limited success rate of current treatment approaches for pulmonary MABC infection, there is an urgent need for novel antimicrobial discovery to combat such emerging pathogens.

2.3. Pseudomonas aeruginosa

Pseudomonas aeruginosa is an aerobic Gram-negative opportunistic pathogen that causes cystic fibrosis, burns wounds, immunodeficiency, and chronic obstructive pulmonary disorder (COPD)[25]. As per a study in European populations, nearly 13% of *P. aeruginosa* isolates demonstrate resistance to multiple antibiotics, making treatment more challenging[26].

The main mechanisms contributing to *P. aeruginosa* resistance involve the over-expression of efflux pumps and the acquisition or mutation of resistance genes. In addition, *P. aeruginosa* gains an acquired resistance mechanism through horizontal gene transfer, particularly genes encoding aminoglycoside-modifying enzymes and beta-lactamases. Furthermore, *P. aeruginosa* reduces antibiotic penetration by decreasing the permeability of the outer membrane, achieved through downregulating and modifying the selectivity of OprD porins[27]. Thus, these mechanisms, in turn, emphasise the importance of innovative strategies to combat resistance and improve treatment outcomes.

2.4. Clostridium difficile

Clostridium difficile is an anaerobic, Gram-positive, spore-forming bacillus that usually causes infections in the colon, leading to healthcare-associated infectious diarrhoea, known as *Clostridium difficile* Infection (CDI). CDI commonly occurs in the community from fecal-oral transmission sources, such as food, compost, manure, zoonotic sources, and other environmental exposures. Broad-spectrum antimicrobials frequently induce this infection by disrupting normal gut flora and allowing *C. difficile* to proliferate. Clinical signs of CDI can range from asymptomatic carriers to mild diarrhoea to severe infections that can lead to sepsis, toxic megacolon, and transmural pancolitis that require colectomy[28]. A meta-analysis in 2023 suggested an upward trend of CDI prevalence in the intensive care units, especially in elderly and critically ill patients[29]. In the United States, *C. difficile* also causes about half a million infections and almost 30,000 yearly deaths[30]. These daunting numbers thus highlight the need for sustainable surveillance of CDI in the healthcare sector by developing protocols to reduce CDI in the community.

C. difficile develops resistance to multiple antimicrobials, particularly clindamycin, cephalosporins, and fluoroquinolones. Besides its naturally refractory spores, *C. difficile* also develops AMR by horizontally acquired resistance genes and *de novo* mutations to drug targets. This enables *C. difficile* to survive under the selection pressures imposed by the antimicrobials[31].

2.5. Staphylococcus aureus

Staphylococcus sp. is commonly linked with increasing bacterial resistance to antimicrobials, such as *Staphylococcus aureus.* This aerobic bacterium is included in the ESKAPE group, a group of bacteria involved in infections and multidrug resistance. They are a critical concern of WHO due to their capacity to acquire, express, and transmit AMR in the healthcare environment, leading to hospital-acquired infections (HAI)[32]. It has evolved resistance mechanisms to many antimicrobial drugs, such as methicillin, the most pronounced drug that *S. aureus* is resistant to[33,34]. The WHO has declared MRSA a priority pathogen, attributed to its superior ability to cause life-threatening diseases. A Klang Valley, Malaysia study suggested community-associated infection may slowly replace hospital-associated MRSA strains. Also, the study found that MRSA contamination is common in dispensing pharmacies in community pharmacies, with a prevalence rate of 22%. This number is concerning since community pharmacies are easily accessible to the public[35]. Thus, all healthcare professionals should practice good hygiene to avoid MRSA infections.

MRSA strains are resistant to beta-lactam antibiotics due to PBP2A. This protein substitutes the normal function of penicillin-binding proteins (PBPs) in cell wall synthesis. PBP2A replaces the transpeptidase function of PBP2 inactivated by beta-lactam antibiotics, allowing the cell to continue building its wall despite the antibiotics. The genes encoding PBP2A are located within the SCC*mec* (staphylococcal chromosomal cassette *mec*) and are transmitted by conjugation or transduction. These characteristics thus contribute to MRSA's ability to spread its resistance and disrupt the normal function of common antibiotics[34].

3. Mechanisms of Bacterial Resistance against Antimicrobial Agents

3.1. Inactivation of Antimicrobial Agents by Bacterial Enzymes

Antibiotic inactivation occurs when the bacterial enzymes inactivate the active antibiotic molecule through two mechanisms: actual degradation of the drug or by transfer of a chemical group[36]. Beta-lactamase, which follows the first mechanism, is a well-studied enzyme produced by bacteria that confers to beta-lactam antibiotics, including penicillin, cephalosporins, carbapenems, and monobactams. These antibiotics exert their bactericidal

effect by inhibiting the penicillin-binding protein (PBP), a pivotal enzyme in cross-linking peptidoglycan strands, disrupting cell wall formation and leading to cell lysis and bacterial death. Thus, beta-lactamase counteracts the action of beta-lactam antibiotics by hydrolysing and cleaving the amide bond in the beta-lactam ring of antibiotics[21]. In addition, bacterial enzymes such as acyltransferase, phosphotransferase, and thioltransferases induce the second mechanism by transferring a chemical group to the drug, such as acetyl, phosphoryl, and adenyl group, rendering them inactive[36,37].

3.2. Reduced Permeability of Antimicrobial Agents

Another mechanism that reduces the intracellular concentration of antimicrobial agents is limiting antibiotic penetration by bacterial cell membranes. *Mycobacterium tuberculosis* possesses a complex lipophilic cell wall impermeable to common antibiotics. Its lipophilic mycolic acid is a barrier that limits the diffusion of hydrophilic antibiotics into bacterial cells. Thus, hydrophilic drugs enter the bacteria via porin channels, which occur more slowly[21,36]. In addition, the lipopolysaccharide (LPS) layer of Gram-negative bacteria contributes to its innate resistance to some antimicrobial agents. *S. aureus* produces a thickened cell wall, which impedes the entrance of vancomycin, leading to an intermediate resistance. For drugs that enter the cell through porin channels, the bacteria limit the drug permeability by downregulation, structural modification, or functional deletion to modify the availability and selectivity of the porin channel[37].

3.3. Insufficient Intracellular Accumulation of Antimicrobial Agents

Bacteria have evolved to reduce the accumulation of antibiotics through the activation of the efflux pump of the cell membrane. Efflux pumps are transport proteins found in the bacterial cell membrane that expel antimicrobial agents from cells, reducing the intracellular concentration below the toxic level and rendering them ineffective[21,36]. Efflux pumps may extrude a single class of antibiotics, such as TetK for tetracyclines and MrsA for macrolides or multiple antibiotic classes. The common multidrug efflux transporters include the resistance-nodulation-cell division (RND) family in Gram-negative bacteria, AcrAB-TolC transporter in *E. coli*, facilitator superfamily (MFS), ATP-binding cassette (ABC) family, and multidrug and toxic-compound extrusion (MATE) family[38].

3.4. Alteration of Antimicrobial Targets Through Enzymatic Modifications

RNA methyltransferase is a class of target-modifying enzymes that modify rRNA elements on the ribosome by adding methyl groups to RNA molecules. This modification causes resistance against ribosomal targeting antimicrobial agents[39,40]. Such modifications

alter the structure and function of the ribosome, thereby weakening the binding of the antibiotic through steric clashes or electrostatic repulsion with methylated nucleotide. Subsequently, it interferes with the antibiotic binding to ribosomes by reducing the affinity of antibiotics for the ribosomes[41]. With that, bacteria evade the inhibitory effects of antibiotics, resuming protein synthesis. For example, ribosomal modification mediated by 16S rRNA methyltransferase, also known as *N*-methyltransferase, methylates N7 of guanine and N1 of adenine. Methylation of N7 on G1405 confers resistance to 4,6-disubstituted aminoglycosides, whereas methylation of N1 on A1408 confers resistance to both 4,6- and 4,5-disubstituted aminoglycosides[41,42].

3.5. Biofilm Formation

Biofilm comprises homogenous or heterogeneous microbial communities living in a self-produced matrix of polymeric substances (EPS) under stressful conditions. Goel *et al*.[43] have suggested that the microbes in this system develop resistance to antimicrobials through different mechanisms, such as poor antibiotic penetration, reduced growth rate, and horizontal gene transfer.

The EPS in biofilm provides a physical barrier against antimicrobial agents by inactivating or entrapping the agents by the matrix. EPS matrix forms chelation with antibiotics, and its enzymes degrade the antibiotic in the matrix. Hence, insufficient antibiotic accumulation in the bacteria allows for a therapeutic effect[43]. Antimicrobial concentrations up to 4 times the minimal inhibitory concentration are necessitated to inhibit the microorganism[44]. In addition, the penetration of positively charged antibiotics may be impeded by the negative component of the biofilm[45].

Furthermore, horizontal gene transfer was reported to be one of the resistance mechanisms against antimicrobials. Since biofilm is a pool of genetic elements produced from the cell lysis of heterogeneous species, this allows an ideal environment for the uptake of resistance species and the exchange of plasmids via conjugation among heterogeneous species[42,46]. Moreover, microorganisms in biofilms may experience nutrient and oxygen depletion due to the high density of the population and reduced diffusion of nutrients through EPS. Thus, such an extreme situation induces physiological changes in bacteria, leading to a stationary growth phase. In this stage, the microorganisms resist the antimicrobials in the biofilm[43].

4. Development of New Generation Antibiotics in Recent 5 Years

Our current clinical development of antibiotics is failing with an exponential decline with newly developed and approved antibiotics over the last three decades. At the same time, the financial burden associated with antibiotic-resistant infections is also skyrocketing, with an annual cost of USD 100 trillion by 2050[10]. Hence, such a phenomenon forces a global rethink to research exploring alternatives to traditional antibiotics, such as next-generation antimicrobials.

NGAs are defined by Gadar and McCarthy as substances that target bacterial virulence components to inhibit pathogenicity without affecting bacterial viability. By deactivating the key virulence components needed for infection establishment, NGAs increase bacteria's vulnerability to clearance by the immune system, thus increasing the susceptibility to traditional antibiotics[47]. On the other hand, Shim[48] suggested that NGAs should be differentiated from conventional antibiotics from three perspectives: evolvability, specificity, or non-immunogenicity. Evolvability allows for the continuous improvement of NGAs in response to bacterial adaptions aimed at countering or evading these antimicrobial agents. Specificity enables the antimicrobial agents to have minimal unintended impact on human microbiota. Non-immunogenicity minimises the adverse effects on human cells and tissues during antimicrobial therapy[48].

4.1. Meropenem-vaborbactam

Since the discovery of antibiotics in the early 20th century, many drug-resistant infections have been reported, including carbapenem-resistant *Enterobacteriaceae* (CRE). It is one of the most common causes of infection that increases the mortality rate worldwide. *Klebsiella pneumoniae* carbapenemase (KPC)-producing organisms are Gram-negative bacteria that produce carbapenemases that destroy the beta-lactam antibiotics such as carbapenems, often serving as the last line of treatment[49]. In August 2017, meropenem-vaborbactam was approved by the Food and Drug Administration (FDA) to treat CRE infections[50]. Meropenem-vaborbactam is a combination of beta-lactam antibiotics, meropenem, and beta-lactamase inhibitor which is vaborbactam. Vaborbactam with cyclic boronic acid-based beta-lactamase inhibitor increases the susceptibility of beta-lactamases-producing microorganisms to antibiotics.

A study done by Novelli A *et al*.[51] shows that vaborbactam effect of the beta-lactamase inhibitor was increased after the addition of 2-thienyl acetyl group. Generally, its mechanism of action involves preventing the serine beta-lactamases, Ambler class A and C enzymes, and the KPC enzyme. It enters the membrane of *K. pneumoniae* with the help of

OmpK35 and OmpK36 porins. Hence, meropenem activity can significantly increase when combined with vaborbactam, especially towards KPC and CRE. In a clinical surveillance study of 991 cases of KPC-producing organisms, meropenem-vaborbactam shows effective results with 0.12 mg/L and 1 mg/L for MIC_{50} and $MIC_{90,}$ respectively[52]. In a study focusing on Targeting Antibiotic Non-susceptible Gram-negative Organism (TANGO), meropenem-vaborbactam was one of the studied agents. Meropenem-vaborbactam was being compared with Piperacillin-tazobactam in a phase III study of TANGO. In terms of microbiological modified intent-to-treat population (m-MITT), meropenem-vaborbactam showed 98.44% whereas piperacillin-tazobactam showed 93.44% of participants who achieved overall success after intravenous treatment. Moreover, 66.67% and 57.69% of participants for meropenem-vaborbactam and piperacillin-tazobactam achieved eradication at fifteen to twenty-three days of treatment. The evaluation of the third primary outcome showed 66.29% of patients with meropenem-vaborbactam and 60.36% with piperacillin-tazobactam that achieved eradication in the microbiological evaluable (ME). Hence, these numbers showed that meropenem-vaborbactam was superior to piperacillin-tazobactam in treating complicated urinary tract infections (cUTI)[53].

4.2. Plazomicin

Plazomicin, also known as ACHN-490, was approved by the FDA in June 2018. It is mainly used to treat cUTI. Although recently developed drugs such as meropenem-vaborbactam and ceftazidime-avibactam can treat CRE, both drugs have no activity on the metallo-beta-lactamases and some of the CRE. From a chemical perspective, plazomicin possesses a structural advantage because it lacks hydroxyl groups in key locations. This prevents it from being deactivated by aminoglycoside-modifying enzymes (AME). Furthermore, the resistance to tobramycin, gentamicin, and amikacin is attributed to the presence of the enzyme N-acetyltransferase AAC (6'). Plazomicin can overcome this resistance with the hydroxyethyl group in its molecule. Besides that, the N-1 position of plazomicin substitutes with 4-amino-2-hydroxybutanoic acid protects the molecule from AAC (3) and ANT (2"). This modification further increases the stability of plazomicin towards pathogens[54]. It is effective against most *Enterobacteriaceae*, including extended-spectrum beta-lactamase (ESBL) and AmpC beta-lactamase (AmpC) producing organisms and most carbapenem-resistant organisms. Most importantly, three of the most common enzymes AAC (3)-II, AAC(6')-I, and ANT(2")-I do not decrease plazomicin activity. Hence, this makes plazomicin a better activity agent than gentamicin and tobramycin, which usually perform with a lower MIC_{90} value.

The effectiveness of plazomicin is tested in a trial known as evaluating plazomicin in cUTI (EPIC). During the phase III trial, the recovery percentage for microbiological modified intent-to-treat (mMITT) is 88% and 91.4% for plazomicin and meropenem respectively. However, plazomicin is superior to meropenem at test-of-cure visit (TOC), which generally ranges from 5 to 12 days after the final dose with 81.7% of plazomicin and 70.1% of meropenem[54,55]. In this case, the microbiological eradication rate for plazomicin is higher than for meropenem. In the CARE (Combating Antibiotic-Resistant *Enterobacteriaceae*) phase III trial, plazomicin-based therapy shows lower ACM (All-Cause Mortality) and Significant Disease-Related Complications (SDRCs) when it is compared to colistin-based therapy with the percentage of 23.5% and 50% respectively at 28th day. In brief, plazomicin, with its excellent efficacy against *Enterobacteriaceae*, including metallo-beta-lactamase-producing *Enterobacteriaceae*, makes it an effective agent that can replace aminoglycosides and other agents.

4.3. Conjugation of Nanoparticles with Antibiotics

The utilisation of engineered nanoparticles is expanding tremendously, including various applications in electronics, renewable energy, and agriculture. Researchers have employed this innovative advancement to combat AMR and generate nanoparticle-antibiotic conjugation. This technology involves attaching antibiotic molecules to nanoparticles, which are tiny particles ranging from 1 to 100 nanometers in size. With their unique physicochemical and biological properties compared to their larger micron-sized counterparts, this innovation enhances the efficacy and delivery of antibiotics[56].

The overall microbiological activity of nanoparticle-antibiotics conjugation involves growth inhibition and lethal cell damage. When metal nanoparticles are in contact with water, they undergo oxidative breakdown, releasing the metal ions. The metal oxide layer dissolves, releasing the metal ions attached to the particle's surface. The released ions react with a series of inorganic and organic substances in the environment, forming several metal ions with different toxicity. For instance, silver ions disrupt the cell membrane and target the cytoplasmic molecules in bacteria. Also, silver ions bind and inhibit the critical metabolic pathway enzymes, inhibiting cell proliferation. This activity releases the Fenton-active Fe2+ ion, which generates radical oxidative species (ROS) that damage bacterial lipids and nucleic acids. This premature electron leakage reacts with the molecular oxygen and generates superoxide radicals[56].

Several cases and studies have proven the potential of various nanoparticles, including silver, gold, and zinc oxide, to enhance the efficacy of antibiotics. Studies found

that applying antibiotics such as erythromycin and vancomycin with silver colloidal has antibacterial activity against Gram-positive bacteria[57]. Moreover, Abo-Shama *et al*.[58] discovered that the conjugation of tetracycline with silver nanoparticles enhanced efficacy against *Salmonella Typhimurium.* Tetracycline accumulates surrounding the cell due to its affinity for the surface of silver nanoparticles. Consequently, this will increase the interaction with the bacterial cell wall and inhibit bacterial growth. In addition, Azizi-Lalabadi M *et al*.[59] suggested that combining zinc oxide with titanium oxide nanoparticles will lead to generating reactive oxidative species such as hydrogen peroxide, superoxide, and other oxygen radicals after exposure to UV irradiation. These harmful radicals and substances will cause oxidative stress, a condition in which the antioxidants of the free radicals are imbalanced. Zinc oxide-nanoparticle was also discovered as an anti-biofilm against MRSA by showing concentration-dependent anti-adherence onto the walls of wells with 65.4 μg/mL. It also breaks down MRSA biofilm at 13.5 μg/mL[60].

A study by Shamaila *et al*.[61] claimed that gold nanoparticles can decrease the affinity of bacterial ribosomes attached by tRNA. This prevents protein synthesis as tRNA does not deliver the amino acid during translation. Besides, they showed that the gold nanoparticles could reduce the adenosine triphosphate (ATP) synthase activity, which functions to synthesise ATP, which is vital in the metabolism of bacteria. Furthermore, they discovered that gold could attach to the thiol group of nicotinamide adenine dinucleotide dehydrogenase (NADH), generating oxidative stress by altering the balance of reduction-oxidation maintained within the bacteria cell[61].

Prophylactic antibiotics, often used in small doses during surgery, can elevate the risk of resistant infections. The risk is heightened when microorganisms form biofilms, leading to chronic infections. This issue can be overcome by incorporating iron oxide nanoparticles into chitosan. This combination can produce ROS through the Fenton reaction, utilising free radicals from iron oxidation[62]. Chitosan-coated iron oxide nanoparticles inhibit the enzyme and undergo metal chelation via electrostatic interaction, demonstrating antimicrobial activity. This is because the negatively charged N-acetyl-muramic acid and sialic acid in the bacterial cell wall interact with positively charged chitosan amino groups[63].

4.4. Macrocyclic Antibiotic

Fidaxomicin is the first drug under this new category of antimicrobial agent. It is a naturally occurring, fermentation-derived 18-member macrocycle. It is a macrocyclic lactone antibiotic drug because fidaxomicin includes an 18-membered lactone ring in its structure.

Unlike macrolides and rifamycin, the antibacterial activity of fidaxomicin is time-dependent rather than concentration-dependent[64].

Fidaxomicin is a narrow-spectrum drug that cures *Clostridium difficile* infection (CDI) and has minimal effect on normal intestinal flora. It is superior to other alternative drugs like vancomycin and metronidazole, primarily due to its significant reduction in the recurrence rate of CDI (13%), making it the ideal CDI treatment[65]. Recurrence is a common and challenging aspect of CDI treatment, with many patients experiencing multiple episodes after initial treatment. This reduction in recurrence improves patient outcomes and decreases the overall burden on healthcare systems by reducing the need for repeated treatments.

Although fidaxomicin's mechanism has yet to be thoroughly studied, current information suggests it inhibits bacterial DNA-dependent RNA polymerase, halting the initiation of bacterial RNA synthesis. Its mechanism has a slight difference compared to rifamycin[66]. Fidaxomicin is minimally absorbed and accumulated in the bloodstream, allowing it to act locally in the gastrointestinal tract to treat CDI precisely[67]. It is effective against Gram-positive bacteria like Clostridia, Staphylococci, and Enterococci. Still, unlike broad-spectrum antibiotics, they do not show any action against beneficial bacteria in the gastrointestinal tract. Hence, fidaxomicin maintains the diversity of the gut microbiota better than other treatments, which helps preserve beneficial bacteria and reduces the likelihood of secondary infections. This safety profile has led to its inclusion in treatment guidelines for CDI due to its specificity and non-immunogenicity.

Additionally, the minimum inhibitory concentration (MIC_{90}) for fidaxomicin is four times lower than for vancomycin, the primary drug of choice for CDI before fidaxomicin's approval[68]. Initially approved by the FDA in 2011 for adult patients over 18 with CDI-associated diarrhoea, fidaxomicin's approved indication was extended in 2020 to include pediatric patients over 6 months old[69]. This extended approval of fidaxomicin demonstrates its versatility across different age groups, further solidifying its role as a superior treatment option for CDI.

4.5. Cephalosporins

Ceftaroline and its prodrug ceftaroline fosamil are recent additions to the cephalosporins category of drugs. It is developed through modification of the 4th generation cephalosporin cefozopran. A phosphonic group is included to enhance the water solubility and facilitate rapid conversion to a bioactive agent. This modification ensures that the drug can be quickly and effectively utilised upon administration. In addition, ceftaroline stands out among the cephalosporins family due to its enhanced activity against MRSA. The

presence of a 1,3-thiazole ring at the 3-position and the amine group in the C7 acyl moiety leads to an increased effectiveness against MRSA[70]. These modifications allow ceftaroline to bind more effectively to PBPs, particularly PBP2a.

Ceftaroline is a beta-lactam antibiotic with a broad activity on Gram-positive and some other Gram-negative bacteria, which includes MRSA and MDR *S. aureus*. However, its effectiveness can be limited by bacteria that express AmpC beta-lactamase and disrupt the antibacterial activity. Like other beta-lactams, ceftaroline interferes with bacterial cell wall synthesis by binding to binding of PBPs[70]. Ceftaroline specifically targets PBP 1-4 and has a higher affinity to PBP2a (mecA), which is associated with methicillin resistance. This trait thus distinguishes ceftaroline from other cephalosporins. In addition, studies have shown that ceftaroline has a high affinity for all 6 PBPs in *S. aureus*. Its action on membrane PBPs of *Enterobacteriaceae* affects both transpeptidase and transglycosidase, leading to higher effectiveness.

Several data have proven the superiority of ceftaroline compared to other antibiotics. In vitro studies demonstrated the high potency of ceftaroline against bacteria strains that are highly resistant to parental cephalosporins, including ceftriaxone[71]. For instance, the MIC of ceftaroline on a spectrum of 120 different cefotaxime-resistant *S. aureus* and lab-cloned R6 strains with PBP mutation is 0.5 μg/ml. Ceftaroline has also presented excellent activity against *S. aureus* strains resistant to amoxicillin, cefotaxime, and penicillin in studies conducted in the United States and Europe. Despite investigations into its potential synergism with other antimicrobial agents, such as vancomycin, these studies suggested that the effectiveness of ceftaroline monotherapy remains comparable to combination therapy[72]. The phase 3 clinical trial of ceftaroline was conducted in 2009, leading to its approval by the NDA. These clinical trials revealed an excellent and consistent safety profile for ceftaroline, comparable to parental cephalosporins[73].

The pharmacokinetic profile of ceftaroline is very similar to other cephalosporins. After an IV administration of 500 mg dose, the plasma concentration (C_{max}) was 16.1 mg/L, the ($AUC_{0\text{-infinity}}$) was 44.8 h.mg/L, the Cmax for the multiple-dose study was 21.3 mg/L, and the AUCss is 56.2 h.mg/L after a 600 g dose every 12h. The distribution in the central and peripheral compartments resulted in a 20% protein binding. Renal clearance is 93.5 mL/min after a 500 mg dose and 118.9 mL/min after the multi-dose study. Studies were done with acute and moderate renal impairment patients as renal excretion drugs are required; the AUC and half-life have shown 25% and 14% increments, respectively[74].

4.6. New generation of glycopeptides

Three novel glycopeptide derivatives, namely oritavancin, dalbavancin, and telavancin, are now being developed to combat the increasing prevalence of vancomycin-resistant bacterial strains. The FDA has authorised all three derivatives following extensive clinical research. They have shown effectiveness against certain drug-resistant bacteria, such as vancomycin-resistant *enterococci* (VRE) and vancomycin-resistant *Staphylococcus aureus* (VRSA), which are typically bacteriostatic[75].

By attaching to the C-terminal D-alanyl D-alanine (D-Ala-D-Ala) of cell wall precursor units, the glycopeptides prevent the formation of cell walls. The N-alkyl-p-chlorophenylbenzyl substituent increases its efficacy on staphylococci resistant to vancomycin and intermediate strains of the antibiotic. In addition, the antimicrobial activity of these glycopeptide derivatives comes from the inhibition of transglycosylation and transpeptidation, which are critical reactions in the biosynthesis of peptidoglycan. The inhibition disrupts the membrane potential by increasing cell permeability, leading to rapid bactericidal action[76].

A meta-analysis has highlighted the superior antimicrobial activity of the novel class of lipoglycopeptides when compared to the classic glycopeptides. This enhanced efficacy is evident in the significantly lower MIC values for lipoglycopeptides against MRSA and VRE. These lower MIC values indicate that smaller amounts of the drug are required to inhibit bacterial growth, which suggests a more potent antibacterial effect[77]. Specifically, dalbavancin showed exceptional potent activity in treating acute bacterial skin and soft-tissue infections, particularly those involving biofilm-forming bacteria. As biofilms are notoriously difficult to eradicate with conventional antibiotics, dalbavancin's potent activity confers a significant advantage over vancomycin, the traditional choice for such infections[78].

All three drugs (dalbavancin, oritavancin, and telavancin) have shown favourable safety profiles and are well tolerated based on multiple clinical trials. These drugs are enhanced by adding a lipophilic side chain to the glycopeptides, extending their half-lives and boosting their activity against Gram-positive cocci. With their long half-lives lasting weeks, dalbavancin and oritavancin require less frequent dosing than telavancin, which has a shorter half-life of 8 hours[79–81]. For dalbavancin, the plasma concentration exceeds the MIC 1 week after administration. Thus, it is being evaluated for a once-a-week dose regimen. Phase II clinical trials show over 90% clinical efficacy, with 1000 mg on the first day and 500 mg on the eighth day[82]. For oritavancin, the long half-life, concentration-dependent bactericidal activity and prolonged half-life have shown that a single dose of oritavancin is non-inferior to repeated doses of vancomycin treatment. Due to its slow tissue elimination, it

also does not require dosage adjustment for renal and hepatic impairment patients[83]. Conversely, telavancin requires dosage adjustment in patients with renal and hepatic impairment, as it is primarily eliminated via the kidneys[84].

4.7. Dual-Acting Immune Antibiotics (DAIAs)

Recent advancements in antimicrobial research have introduced new classes of antimicrobials known as dual-acting immune-antibiotics (DAIAs). They show a synergistic dual activity, effectively targeting the resistant microbes while enhancing the immune response mediated by T cells. This dual action confers a significant advantage, challenging resistance development[85]. DAIAs tackle the methyl-D-erythritol phosphate (MEP) pathway of isoprenoid biosynthesis using IspH enzymes, which are only conserved in Gram-negative bacteria, mycobacteria, and apicomplexans. Notably, the absence of the MEP pathway in humans makes its enzymes ideal targets for developing novel antimicrobial agents[86]. In 2017, the WHO released a list of antibiotic-resistant priority pathogens. This list was designed to guide the development of new antimicrobials, as there is an increasing number of bacteria strains resistant to all existing antibiotics globally. Significantly, 9 out of 12 identified bacteria possess an IspH enzyme, highlighting its potential as a promising target for antibiotic development[87].

By inhibiting IspH, DAIAs impede isoprenoid synthesis, triggering the first mechanism of action. Isoprenoids are critical in eukaryotes as cholesterol for membrane stabiliser, menaquinone or ubiquinone for electron transport in cellular respiration, and bactoprenol as a carrier of the biosynthetic sugar precursors of peptidoglycan. The reduction in isoprenoid levels thus leads to bacterial death. Furthermore, inhibition of IspH leads to an accumulation of (E)-4-Hydroxy-3-methyl-but-2-enyl pyrophosphate (HMBPP) in the bacteria. HMBPP acts as a phosphoantigen (Pags) that activates Vγ9/Vδ2 T cells, a subtype of T lymphocytes in humans[86]. Through mediating innate and adaptive properties against microbes, the Vγ2Vδ2 T cells release cytolytic mediators (granzymes, granzyme, and perforin) and pro-inflammatory cytokines, thereby inducing target cell apoptosis. The subsequent release of cytokines and chemokines activates immune cells to promote an adaptive immune response[88]. Studies have proven that HMBPP is the most potent natural Pag[86]. By acting on IspH, DAIAs have successfully impeded isoprenoid biosynthesis and united the immune system to combat bacteria, leading to better bacterial clearance.

As no IspH inhibitor is available in the market, Jamod *et al*.[89] repurposed 35 immune boosters against the IspH enzyme. Out of which, 4’-fluorouridine was due to its glide score and binding affinity with IspH enzyme by interacting with the catalytic pocket residues of IspH enzyme. The authors suggested that hydrogen bonds, π – π stacking, π - cationic

interactions play the utmost interactions between 4'-FU and the binding pocket. Thus, 4'-FU pharmacophores may be further utilised to develop antimicrobial agents to treat resistant bacterial infections[89].

4.8. Cyclic Diguanylate (c-di-GMP) Inhibitors

Cyclic diguanylate (c-di-GMP) is a nucleotide-based secondary messenger present in bacteria. c-di-GMP selectively binds to various downstream effectors, swiftly modifying their activity in response to environmental signals and coordinating with the effector. This alters bacterial physiology at the transcriptional, translational, or post-translational level[90]. It integrates various physiological functions, from motility and biofilm formation to pathogenicity and secondary metabolite production, particularly in response to stressful situations. For instance, it regulates the transformation between the planktonic and biofilm states. High levels of c-di-GMP promote biofilm formation to protect bacteria from environmental stresses and antimicrobials by increasing adhesins and exopolysaccharide production. In addition, by altering c-di-GMP levels, bacteria adopt different motility in response to environments, promoting survival. These two fundamental properties thus make approaches to disrupt the regulatory cascade of c-di-GMP an attractive target for developing NGAs[91].

Nitric oxide is a c-di-GMP inhibitor in bacteria that creates oxidative stress in the bacterial biofilm, inducing dispersal and preventing motility and adhesion to the host. Nitric oxide achieves this by activating phosphodiesterase, an enzyme that hydrolyses intracellular c-di-GMP, thereby reducing its level[47]. In *P. aeruginosa*, nitric oxide has been shown to significantly decrease biofilm formation and impair bacterial motility[92]. Therapeutically, compounds that activate phosphodiesterase or mimic their action could serve as promising antimicrobial agents. Other bioactive molecules that target c-di-GMP include c-di-GMP receptor antagonists that bind to the allosteric inhibitory site (triazole-linked analogs and 2'-F-c-di-GMP), inhibitors of c-di-GMP biosynthesis (azathioprine) cahuitamycins, diffusible signal factor (DSF)[47].

5. Discussion

While generally acknowledged, the proposed lower selection pressure of NGAs does not imply that they are impervious to resistance. In fact, bacteria may evolve resistance mechanisms against their activity, which is a possibility that warrants further investigation [47]. The bacteria's adaptability is attributed to their remarkable genetic flexibility. In addition, while NGAs are combined with other antimicrobials to reduce resistance development, the risk of developing cross-resistance is still significant. The generation and increased diffusion

of antibiotic-resistance genes (ARGs) in the environment can occur through various pathways, such as mutation, horizontal gene transfer, efflux pumps, and biofilm formation. Thus, the capacity for bacteria to develop mechanisms to overcome their activity must be elucidated thoroughly through laboratory research, genomic studies, and surveillance for the sustainable use of NGAs.

Furthermore, while NGAs are intended to target specific pathogens, the host-microbiome interaction remains unclear because antimicrobials could also interfere with the behaviours of beneficial microorganisms in our microbiome. For instance, NGAs interfere with c-di-GMP, a secondary messenger utilised by bacteria that integrates cellular processes like adhesion, motility, and virulence to halt the signaling cascade in pathogens by disrupting intracellular nucleotide pools[90]. However, it may impair the biofilm-forming ability of normal microbiota to protect and maintain a stable microbiome, increasing the susceptibility of pathogens colonisation. The altered biofilm formation then increases the competition between different species for resources. An impaired microbiome environment thus affects metabolic pathways like fat digestion and absorption, leading to nutrient deficiency. Hence, there is an urgent need for further research to investigate the long-term effects of antibiotic usage on the variety and stability of the microbiome[93].

Insufficient knowledge of the pharmacokinetics and pharmacodynamics profile of NGAs in different patient populations has drawn the attention of more detailed studies on drug metabolism and interactions. Further investigation is required to examine the absorption of novel antibiotics in various patient populations, particularly those with medical problems that may impact drug absorption. Also, comprehensive surveillance systems are crucial for tackling AMR trends and guiding interventions[94]. However, gaps exist in data collection, especially in low- and middle-income countries.

Addressing AMR requires a collaborative effort from different sectors and stakeholders but is fraught with challenges and limitations. While efforts to address AMR have been made globally, studies conducted by Caudell *et al.*[95] and Mangesho *et al.*[96] suggested that one of the fundamental challenges in tackling AMR is the knowledge-practice gap within low- and middle-income countries (LMICs). Such a gap suggested a significant discrepancy between the community's knowledge and attitudes toward AMR. Despite AMR being recognised as a consequence of inappropriate antimicrobial use, the community fails to translate this awareness into sensible antimicrobial use. The root causes of this phenomenon are multifaceted, including limited access to healthcare and diagnostic facilities, socioeconomic factors, and low health literacy. As the community in LMICs is constrained by limited resources, patients may rely on physiological bias, that is, dependent on the

preference for short-term rewards over the long-term consequences of antibiotic misuse. In addition, informal drug shops often flourish in LMICs, which have limited access to formal healthcare. This enables easy access to antibiotics without proper prescriptions[96]. Thus, merely addressing knowledge deficits is insufficient to change the behaviour towards AMR[97].

The use of antibiotics in agriculture, livestock, and animal husbandry presents significant challenges in the fight against AMR transmission and development. The overuse and misuse of antimicrobials often create a breeding ground for resistant bacteria as they are commonly administered for therapeutic purposes, infection prevention, and animal growth promotions[98]. While antibiotics have proven beneficial in these industries, their widespread and indiscriminate use has severe repercussions for both human health and the environment. For instance, antibiotics administered to animals as growth promoters may develop selective pressure, leading to the selection of resistant bacterial strains within the animal's gut microbiota. This leads to the development and proliferation of antibiotic-resistant strains. Worst still, these antibiotics may not be wholly absorbed in the gut. They can be excreted as waste, contaminating soil and water when they serve as manure. This contamination transfers the traces of antibiotic or resistant bacterial strains to the soil ecosystem and plants, affecting the soil microbiota and developing AMR among soil bacteria and crops. Furthermore, antibiotics or resistant strains may diffuse along the waterways and soil, further amplifying the risk of AMR transmission to humans, animals, and environments[99]. High levels of antimicrobial residues and resistant strains have been found in foods of animal origin, such as eggs, chicken and beef meat, and raw milk[100]. This highlights the potential for horizontal gene transfer from the environments to animals and humans, contributing to the emergence of AMR due to improper antimicrobial management[98].

6. Conclusion

Meropenem-vaborbactam has shown crucial efficacy against CRE, which produces KPC compared to piperacillin-tazobactam. The addition of vaborbactam enhances meropenem's activity against beta-lactamase-producing microorganisms. Clinical studies demonstrated its superiority over piperacillin-tazobactam, making it a valuable option for treating cUTI. Approved in 2018, plazomicin tackles metallo-beta-lactamases and specific CRE as its structural modifications protect it from aminoglycoside-modifying enzymes and enzyme N-acetyltransferase AAC, making it effective where other aminoglycosides fail. Plazomicin demonstrated better antimicrobial activity than meropenem and lower all-cause mortality and disease-related complications compared to colistin-based therapies, solidifying its position in treating resistant infections. Nanoparticle-antibiotic conjugates solve the rising

problem of antimicrobial resistance worldwide by leveraging the unique properties of nanoparticles. It has been evidenced by studies that nanotechnology demonstrated significant antibacterial activity with silver, gold, and zinc oxide. These conjugates disrupt bacterial cell membranes, generate ROS, and inhibit bacterial enzymes, offering a promising strategy against antimicrobial resistance. Fidaxomicin is a new agent that tackles CDI by inhibiting the DNA-dependent RNA polymerase with minimal impact on normal intestinal flora. Its narrow-spectrum activity and low recurrence rates of CDI make it superior to vancomycin and metronidazole.

A fifth-generation cephalosporin, ceftaroline has an almost similar mechanism of action to previous beta-lactam antibiotics. However, it is more notable against MRSA. Its structural modifications enhance binding to PBP2a, which is associated with methicillin resistance. As such, its activity and safety profile make it a critical highlight to the cephalosporin family. The lipoglycopeptides – oritavancin, dalbavancin, and telavancin have bacteriostatic effects on VRE and VRSA. They exhibit enhanced efficacy due to structural modifications that allow binding to critical cell wall precursors. DAIAs represent a novel approach that targets the IspH enzyme in the MEP pathway, which is unique to bacteria and absent in humans. DAIAs offer a dual mechanism by inhibiting isoprenoid synthesis and enhancing T-cell immune responses to prevent resistance. Notably, 4’-flurouridine has shown promise as an IspH enzyme inhibitor, leading to adequate bacterial clearance. c-di-GMP modulation, including nitric oxide and other inhibitors, offers potent strategies to disrupt biofilm formation and bacterial motility. Additional strategies include c-di-GMP receptor antagonists and biosynthesis inhibitors, highlighting diverse approaches to combat AMR by targeting bacterial virulence pathways.

These advanced medications and novel approaches are essential in tackling the worldwide issue of antimicrobial resistance. Each approach provides distinct methods and benefits, contributing to a diverse strategy for addressing resistant illness. Ongoing research and development in these fields6 are crucial to guarantee the availability of viable medicines in response to the changing microbial challenges.

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References

1. Sabbatani S, Catena F, Ansaloni L. The Long and Dramatic History of Surgical Infections. Arch Med (Oviedo) 2016; 08(06): 1-9. doi: 10.21767/1989-5216.1000173
2. Al-Worafi YM. Antibiotics safety issues. In: Drug Safety in Developing Countries. Sana'a, Yemen: Elsevier Academic Press; 2020. p. 87–103. doi: 10.1016/C2019-0-00778-9
3. Ribeiro da Cunha, Fonseca, Calado. Antibiotic Discovery: Where Have We Come from, Where Do We Go?. Antibiotics 2019; 8(2): 45. doi: 10.3390/antibiotics8020045
4. Sagar S, Kaistha S, Das AJ, Kumar R. Emergence of Antibiotic-Resistant Microbes Immediately After the Discovery of Antibiotics. In: Antibiotic Resistant Bacteria: A Challenge to Modern Medicine. Singapore: Springer Singapore; 2019. p. 15–30. doi: 10.1007/978-981-13-9879-7_2
5. World Health Organization. Antimicrobial resistance. 2017. [cited 30 June 2024]. Available from: https://www.who.int/news-room/questions-and-answers/item/antimicrobial-resistance#:~:text=Antimicrobial%20resistance%20occurs%20naturally%20but%20is%20facilitated%20by,encourage%20the%20development%20and%20spread%20of%20drug%20resistance.
6. Ahmed SK, Hussein S, Qurbani K, *et al*. Antimicrobial resistance: Impacts, challenges, and future prospects. J Med Surg Public Health 2024; 2: 100081. doi: 10.1016/j.glmedi.2024.100081
7. Dadgostar P. Antimicrobial resistance: implications and costs. Infect Drug Resist 2019; 12: 3903–10. doi: 10.2147/IDR.S234610.
8. Cong Y, Yang S, Rao X. Vancomycin resistant Staphylococcus aureus infections: A review of case updating and clinical features. J Adv Res 2020; 21: 169–76. doi: 10.1016/j.jare.2019.10.005
9. Nelson RE, Hatfield KM, Wolford H, *et al*. National Estimates of Healthcare Costs Associated With Multidrug-Resistant Bacterial Infections Among Hospitalised Patients in the United States. Clin Infect Dis 2021; 72(Supplement_1): S17–26. doi: 10.1093/cid/ciaa1581
10. World Bank Group. Drug-resistant infections: A Threat to Our Economic Future. 2017. [cited 30 June 2024]. Available from: https://www.worldbank.org/en/topic/health/publication/drug-resistant-infections-a-threat-to-our-economic-future
11. Centers for Disease Control and Prevention. Antibiotic resistance threats in the United States. 2019. [cited 30 June 2024]. Available from: https://stacks.cdc.gov/view/cdc/82532
12. Nanayakkara AK, Boucher HW, Fowler VG, *et al*. Antibiotic resistance in the patient with cancer: Escalating challenges and paths forward. CA Cancer J Clin 2021;71(6):488–504. doi: 10.3322/caac.21697
13. Ibrahim S, Al-Saryi N, Al-Kadmy IMS, *et al*. Multidrug-resistant Acinetobacter baumannii as an emerging concern in hospitals. Mol Biol Rep 2021; 48(10): 6987–98. doi: 10.1007/s11033-021-06690-6
14. Ghimire U, Kandel R, Neupane M, *et al*. Biofilm Formation and blaOXA Genes Detection Among Acinetobacter baumannii from Clinical Isolates in a Tertiary Care Kirtipur Hospital, Nepal. Prog Microbes Mol Biol 2021; 4(1). doi: 10.36877/pmmb.a0000245
15. Vázquez-López R, Solano-Gálvez SG, Juárez Vignon-Whaley JJ, *et al*. Acinetobacter baumannii Resistance: A Real Challenge for Clinicians. Antibiotics 2020 23; 9(4): 205. doi: 10.3390/antibiotics9040205

16. Kyriakidis I, Vasileiou E, Pana ZD, *et al*. Acinetobacter baumannii Antibiotic Resistance Mechanisms. Pathogens 2021; 10(3): 373. doi: 10.3390/pathogens10030373
17. Seleim SM, Mostafa MS, Ouda NH, *et al*. The role of pmrCAB genes in colistin-resistant Acinetobacter baumannii. Sci Rep 2022; 12(1): 20951. doi: 10.1038/s41598-022-25226-x
18. Mancuso G, Midiri A, Gerace E, *et al*. Bacterial Antibiotic Resistance: The Most Critical Pathogens. Pathogens 2021; 10(10): 1310. doi: 10.3390/pathogens10101310
19. Victoria L, Gupta A, Gómez JL, *et al*. Mycobacterium abscessus complex: A Review of Recent Developments in an Emerging Pathogen. Front Cell Infect Microbiol 2021; 11: 659997. doi: 10.3389/fcimb.2021.659997
20. Recchia D, Stelitano G, Stamilla A, *et al*. Mycobacterium abscessus Infections in Cystic Fibrosis Individuals: A Review on Therapeutic Options. Int J Mol Sci 2023; 24(5): 4635. doi: 10.3390/ijms24054635
21. Pulingam T, Parumasivam T, Gazzali AM, *et al*. Antimicrobial resistance: Prevalence, economic burden, mechanisms of resistance and strategies to overcome. Eu J Pharm Sci 2022; 170: 106103. doi: 10.1016/j.ejps.2021.106103
22. Richard M, Gutiérrez AV, Kremer L. Dissecting erm(41)-Mediated Macrolide-Inducible Resistance in Mycobacterium abscessus. Antimicrob Agents Chemother 2020; 53(4): 1367-76. doi: 10.1128/AAC.01275-08.
23. Guo Q, Chen J, Zhang S, *et al*. Efflux Pumps Contribute to Intrinsic Clarithromycin Resistance in Clinical, Mycobacterium abscessus Isolates. Infect Drug Resist 2020; 13: 447–54. doi: 10.2147/IDR.S239850
24. Gorzynski M, Week T, Jaramillo T, *et al*. Mycobacterium abscessus Genetic Determinants Associated with the Intrinsic Resistance to Antibiotics. Microorganisms 2021; 9(12): 2527. doi: 10.3390/microorganisms9122527.
25. Langendonk RF, Neill DR, Fothergill JL. The Building Blocks of Antimicrobial Resistance in Pseudomonas aeruginosa: Implications for Current Resistance-Breaking Therapies. Front Cell Infect Microbiol. 2021; 11: 665759. doi: 10.3389/fcimb.2021.665759.
26. Botelho J, Grosso F, Peixe L. Antibiotic resistance in Pseudomonas aeruginosa – Mechanisms, epidemiology and evolution. Drug Resist Updat 2019; 44: 100640. doi: 10.1016/j.drup.2019.07.002.
27. Qin S, Xiao W, Zhou C, *et al*. Pseudomonas aeruginosa: pathogenesis, virulence factors, antibiotic resistance, interaction with host, technology advances and emerging therapeutics. Signal Transduct Target Ther 2022; 7(1): 199. doi: 10.1038/s41392-022-01056-1.
28. De Roo AC, Regenbogen SE. Clostridium difficile Infection: An Epidemiology Update. Clin Colon Rectal Surg 2020; 33(02): 49–57. doi: 10.1055/s-0040-1701229.
29. Balsells E, Shi T, Leese C, *et al*. Global burden of Clostridium difficile infections: a systematic review and meta-analysis. J Glob Health 2019; 9(1): 010407. doi: 10.7189/jogh.09.010407
30. Lessa FC, Mu Y, Bamberg WM, *et al*. Burden of Clostridium difficile Infection in the United States. N Engl J Med 2015; 372(9): 825–34. doi: 10.1056/NEJMoa1408913.
31. Dureja C, Olaitan AO, Hurdle JG. Mechanisms and impact of antimicrobial resistance in Clostridioides difficile. Curr Opin Microbiol 2022; 66: 63–72. doi: 10.1016/j.mib.2022.01.004.

32. Rizki LP, Murni IK, Aman AT, *et al*. Environmental Metagenomic Analysis of "ESKAPE" Pathogens in the Pediatric Intensive Care Unit of General Hospital Yogyakarta Indonesia. Prog Microbes Mol Biol 2024; 7(1). doi: 10.36877/pmmb.a0000398
33. Nandhini P, Kumar P, Mickymaray S, *et al*. Recent Developments in Methicillin-Resistant Staphylococcus aureus (MRSA) Treatment: A Review. Vol. 11, Antibiotics 2022; 11(5): 606. doi: 10.3390/antibiotics11050606
34. Mlynarczyk-Bonikowska B, Kowalewski C, Krolak-Ulinska A, *et al*. Molecular Mechanisms of Drug Resistance in Staphylococcus aureus. Int J Mol Sci 2022; 23(15): 8088. doi: 10.3390/ijms23158088.
35. Chow YL, Tham HW. Methicillin-resistant Staphylococcus aureus (MRSA) on dispensing counters of community pharmacies in Klang Valley. Prog Microbes Mol Bio 2020; 3(1): a0000084. doi: pmmb.a0000084
36. Abushaheen MA, Muzaheed, Fatani AJ, *et al*. Antimicrobial resistance, mechanisms and its clinical significance. Dis Mon 2020; 66(6): 100971. doi: 10.1016/j.disamonth.2020.100971.
37. C Reygaert W. An overview of the antimicrobial resistance mechanisms of bacteria. AIMS Microbiol 2018; 4(3): 482–501. doi: 10.3934/microbiol.2018.3.482
38. Lamut A, Peterlin Mašič L, Kikelj D, *et al*. Efflux pump inhibitors of clinically relevant multidrug resistant bacteria. Med Res Rev 2019; 39(6): 2460–504. doi: 10.1002/med.21591.
39. Qiu L, Jing Q, Li Y, *et al*. RNA modification: mechanisms and therapeutic targets. Mol Biomed 2023; 4(1): 25. doi: 10.1016/j.molmed.2020.05.001
40. Schaenzer AJ, Wright GD. Antibiotic Resistance by Enzymatic Modification of Antibiotic Targets. Trends Mol Med 2020; 26(8): 768–82. doi: 10.1186/s43556-023-00139-x
41. Wachino JI, Doi Y, Arakawa Y. Aminoglycoside Resistance. Infect Dis Clin North Am 2020; 34(4): 887–902. doi: 10.1016/j.idc.2020.06.002.
42. Schaenzer AJ, Wright GD. Antibiotic Resistance by Enzymatic Modification of Antibiotic Targets. Trends Mol Med 2020; 26(8): 768–82. doi: 10.1016/j.molmed.2020.05.001.
43. Goel N, Fatima SW, Kumar S, *et al*. Antimicrobial resistance in biofilms: Exploring marine actinobacteria as a potential source of antibiotics and biofilm inhibitors. Biotechnol Rep 2021; 30: e00613. doi: 10.1016/j.btre.2021.e00613.
44. Grande R, Puca V, Muraro R. Antibiotic resistance and bacterial biofilm. Expert Opin Ther Pat 2020; 30(12): 897–900. doi: 10.1080/13543776.2020.1830060.
45. Ciofu O, Moser C, Jensen PØ, *et al*. Tolerance and resistance of microbial biofilms. Nat Rev Microbiol 2022; 20(10): 621–35. doi: 10.1038/s41579-022-00682-4.
46. Bowler P, Murphy C, Wolcott R. Biofilm exacerbates antibiotic resistance: Is this a current oversight in antimicrobial stewardship? Antimicrob Resist Infect Control 2020; 9(1): 162. doi: 10.1186/s13756-020-00830-6.
47. Gadar K, McCarthy RR. Using next generation antimicrobials to target the mechanisms of infection. NPJ Antimicrob Resist 2023; 1(1): 11. doi: 10.1038/s44259-023-00011-6
48. Shim H. Three Innovations of Next-Generation Antibiotics: Evolvability, Specificity, and Non-Immunogenicity. Antibiotics 2023; 12(2): 204. doi: 10.3390/antibiotics12020204.

49. Reyes J, Aguilar AC, Caicedo A. Carbapenem-Resistant Klebsiella pneumoniae: Microbiology Key Points for Clinical Practice. Int J Gen Med 2019; 12: 437–46. doi: 10.2147/IJGM.S214305.
50. Lee Y, Kim J, Trinh S. Meropenem-Vaborbactam (VabomereTM): Another Option for Carbapenem-Resistant Enterobacteriaceae. P T 2019; 44(3): 110–3.
51. Novelli A, Del Giacomo P, Rossolini GM, *et al*. Meropenem/vaborbactam: a next generation β-lactam β-lactamase inhibitor combination. Expert Rev Anti Infect Ther 2020; 18(7): 643–55. doi: 10.1080/14787210.2020.1756775.
52. Hackel MA, Lomovskaya O, Dudley MN, *et al*. In Vitro Activity of Meropenem-Vaborbactam against Clinical Isolates of KPC-Positive Enterobacteriaceae. Antimicrob Agents Chemother 2017; 62(1): e01904-17. doi: 10.1128/AAC.01904-17.
53. Lee YR, Baker NT. Meropenem-vaborbactam: a carbapenem and beta-lactamase inhibitor with activity against carbapenem-resistant Enterobacteriaceae. *Eur J Clin Microbiol Infect Dis 2018; 37*(8), 1411–19. doi: 10.1007/s10096-018-3260-4
54. Wang N, Luo J, Deng F, *et al*. Antibiotic Combination Therapy: A Strategy to Overcome Bacterial Resistance to Aminoglycoside Antibiotics. Front Pharmacol 2022; 13: 839808. doi: 10.3389/fphar.2022.839808.
55. Eljaaly K, Alharbi A, Alshehri S, *et al*. Plazomicin: A Novel Aminoglycoside for the Treatment of Resistant Gram-Negative Bacterial Infections. Drugs 2019; 79(3): 243–69. doi: 10.1007/s40265-019-1054-3.
56. Yonathan K, Mann R, Mahbub KR, *et al*. The impact of silver nanoparticles on microbial communities and antibiotic resistance determinants in the environment. Environ Pollut 2022; 293: 118506. doi: 10.1016/j.envpol.2021.118506
57. Jelinkova P, Mazumdar A, Sur VP, *et al*. Nanoparticle-drug conjugates treating bacterial infections. J Control Release 2019; 307: 166–85. oi: 10.1016/j.jconrel.2019.06.013.
58. Abo-Shama UH, El-Gendy H, Mousa WS, *et al*. Synergistic and Antagonistic Effects of Metal Nanoparticles in Combination with Antibiotics Against Some Reference Strains of Pathogenic Microorganisms. Infect Drug Resist 2020; 13: 351–62. doi: 10.2147/IDR.S234425.
59. Azizi-Lalabadi M, Ehsani A, Divband B, *et al*. Antimicrobial activity of Titanium dioxide and Zinc oxide nanoparticles supported in 4A zeolite and evaluation the morphological characteristic. Sci Rep 2019; 9(1): 17439. doi: 10.1038/s41598-019-54025-0.
60. Kemung HM, Tan LTH, Khaw KY, *et al*. An Optimised Anti-adherence and Anti-biofilm Assay: Case Study of Zinc Oxide Nanoparticles versus MRSA Biofilm. Prog Microbes Mol Biol 2020; 3(1). doi: 10.36877/pmmb.a0000091
61. Shamaila S, Zafar N, Riaz S, *et al*. Gold Nanoparticles: An Efficient Antimicrobial Agent against Enteric Bacterial Human Pathogen. Nanomaterials 2016; 6(4): 71. doi: 10.3390/nano6040071
62. Saravanakumar K, Sathiyaseelan A, Manivasagan P, *et al*. Photothermally responsive chitosan-coated iron oxide nanoparticles for enhanced eradication of bacterial biofilms. Biomat Adv 2022; 141: 213129. doi: 10.1016/j.bioadv.2022.213129

63. Shrifian-Esfahni A, Salehi M, Nasr-Esfahni M, *et al*. Chitosan-modified superparamgnetic iron oxide nanoparticles: Design, fabrication, characterisation and antibacterial activity. Chemik 2015; 69(1): 19-32.
64. National Center for Biotechnology Information. PubChem Compound Summary for CID 10034073, Fidaxomicin. 2024. [cited 30 June 2024]. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/Tiacumicin-B
65. Polivkova S, Krutova M, Capek V, *et al*. Fidaxomicin versus metronidazole, vancomycin and their combination for initial episode, first recurrence and severe Clostridioides difficile infection — An observational cohort study. Int J Infect Dis 2021; 103: 226–33. doi: 10.1016/j.ijid.2020.11.004
66. Dorst A, Berg R, Gertzen CGW, *et al*. Semisynthetic Analogs of the Antibiotic Fidaxomicin—Design, Synthesis, and Biological Evaluation. ACS Med Chem Lett 2020; 11(12): 2414–20. doi: 10.1021/acs.joc.8b00101
67. Cerner M. Fidaxomicin. 2024. [cited 30 June 2024]. Available from: https://www.drugs.com/mtm/fidaxomicin.html
68. Dailler D, Dorst A, Schäfle D, *et al*. Novel fidaxomicin antibiotics through site-selective catalysis. Commun Chem 2021; 10; 4(1): 59. doi: 10.1038/s42004-021-00501-6.
69. Food Drug Adminstration. DIFCID Prescribing Information. 2020. [cited 30 June 2024]. Available from: https://www.accessdata.fda.gov/drugsatfda_docs/label/2020/213138lbl.pdf
70. Lounsbury N, Reeber M, Mina G, *et al*. A Mini-Review on Ceftaroline in Bacteremia Patients with Methicillin-Resistant Staphylococcus aureus (MRSA) Infections. Antibiotics 2019; 8(1): 30. doi: 10.3390/antibiotics8010030.
71. Eljaaly K, Wali H, Basilim A, *et al*. Clinical cure with ceftriaxone versus ceftaroline or ceftobiprole in the treatment of staphylococcal pneumonia: a systematic review and meta-analysis. Int J Antimicrob Agents. 2019; 54(2): 149–53. doi: 10.1016/j.ijantimicag.2019.05.023.
72. Tsai CE, Yang CJ, Chuang YC, *et al*. Evaluation of the synergistic effect of ceftaroline against methicillin-resistant Staphylococcus aureus. Int J Infect Dis 2022; 122: 230–6. doi: 10.1016/j.ijid.2022.05.057
73. American Society of Health-System Pharmacists. Ceftaroline Fosamil Monograph for Professionals. 2020. [cited 30 June 2024]. Available from: https://www.drugs.com/monograph/ceftaroline.html
74. Laudano JB. Ceftaroline fosamil: a new broad-spectrum cephalosporin. J Antimicrobial Chemother 2011; 66 Suppl 3:iii11-8. doi: 10.1093/jac/dkr095.
75. Rai J, Randhawa G, Kaur M. Recent advances in antibacterial drugs. Int J Appl Basic Med Res 2013;3(1):3. doi: 10.4103/2229-516X.112229.
76. Sarkar P, Haldar J. Glycopeptide Antibiotics. In: Antibiotic Drug Resistance. Karnataka, India: Wiley; 2019. p. 73–95.
77. Liu F, Rajabi S, Shi C, *et al*. Antibacterial activity of recently approved antibiotics against methicillin-resistant Staphylococcus aureus (MRSA) strains: A systematic review and meta-analysis. Ann Clin Microbiol Antimicrob 2022; 21(1): 37. doi: 10.1186/s12941-022-00529-z.

78. Di Pilato V, Ceccherini F, Sennati S, *et al*. In vitro time-kill kinetics of dalbavancin against Staphylococcus spp. biofilms over prolonged exposure times. Diagn Microbiol Infect Dis 2020; 96(2): 114901. doi: 10.1016/j.diagmicrobio.2019.114901
79. Fong IW. New Glycopeptides: Telavancin, Dalbavancin, and Oritavancin. In: New Antimicrobials: For the Present and the Future. Toronto, Canada: Springer, Cham; 2023. p. 59–75.
80. Rose WE, Hutson PR. A Two-Dose Oritavancin Regimen Using Pharmacokinetic Estimation Analysis. Drugs Real World Outcomes 2020; 7(Suppl 1): 36–40. doi: 10.1007/s40801-020-00188-6.
81. Molina KC, Miller MA, Mueller SW, *et al*. Clinical Pharmacokinetics and Pharmacodynamics of Dalbavancin. Clin Pharmacokinet 2022; 61(3): 363–74. doi: 10.1007/s40262-021-01088-w.
82. Daum RS. Staphylococcus aureus. In: Principles and Practice of Pediatric Infectious Diseases. Philadelphia, Pennsylvania: Elsevier; 2018. p. 692-706.e4.
83. Baiardi G, Cameran Caviglia M, Piras F, *et al*. The Clinical Efficacy of Multidose Oritavancin: A Systematic Review. Antibiotics 2023; 12(10): 1498. doi: 10.3390/antibiotics12101498
84. Wu W, Ke M, Ye L, *et al*. Application of physiologically based pharmacokinetic modeling to predict the pharmacokinetics of telavancin in obesity with renal impairment. Eur J Clin Pharmacol 2021; 77(7): 989–98. doi: 10.1007/s00228-020-03072-y.
85. Chinemerem Nwobodo D, Ugwu MC, Oliseloke Anie C, *et al*. Antibiotic resistance: The challenges and some emerging strategies for tackling a global menace. J Clin Lab Anal 2022; 36(9): e24655. doi: 10.1002/jcla.24655.
86. Allamand A, Piechowiak T, Lièvremont D, *et al*. The Multifaceted MEP Pathway: Towards New Therapeutic Perspectives. Molecules 2023; 28(3): 1403. doi: 10.3390/molecules28031403.
87. Jobelius H, Bianchino G, Borel F, *et al*. The Reductive Dehydroxylation Catalyzed by IspH, a Source of Inspiration for the Development of Novel Anti-Infectives. Molecules 2022; 27(3): 708. doi: 10.3390/molecules27030708.
88. Gay L, Mezouar S, Cano C, *et al*. Role of Vγ9vδ2 T lymphocytes in infectious diseases. Front Immunol 2022; 13: 928441. doi: 10.3389/fimmu.2022.928441.
89. Jamod H, Mehta K, Sakariya A, *et al*. Dual Acting Immuno-Antibiotics: Computational Investigation on Antibacterial Efficacy of Immune Boosters Against Isoprenoid H Enzyme. Assay Drug Dev Technol 2022; 20(5): 225–36. doi: 10.1089/adt.2022.038.
90. Wang Z, Song L, Liu X, *et al*. Bacterial second messenger c-di-GMP: Emerging functions in stress resistance. Microbiol Res 2023; 268: 127302. doi: 10.1016/j.micres.2023.127302
91. Jenal U, Reinders A, Lori C. Cyclic di-GMP: second messenger extraordinaire. Nat Rev Microbiol 2017; 15(5): 271–84. doi: 10.1038/nrmicro.2016.190.
92. Howlin RP, Cathie K, Hall-Stoodley L, *et al*. Low-Dose Nitric Oxide as Targeted Anti-biofilm Adjunctive Therapy to Treat Chronic Pseudomonas aeruginosa Infection in Cystic Fibrosis. Mol Ther 2017; 25(9): 2104–16. doi: 10.1016/j.ymthe.2017.06.021
93. Yoon MY, Yoon SS. Disruption of the Gut Ecosystem by Antibiotics. Yonsei Med J 2018;59(1):4. doi: doi.org/10.3349/ymj.2018.59.1.4
94. Kuai YH, Law JWF, Ong YS, *et al*. Role of SarA in Staphylococcus aureus: A Virulence Target For Therapeutic Strategies. Prog Microbes Mol Biol 2024;7(1). doi: 10.36877/pmmb.a0000444

95. Caudell MA, Dorado-Garcia A, Eckford S, *et al*. Towards a bottom-up understanding of antimicrobial use and resistance on the farm: A knowledge, attitudes, and practices survey across livestock systems in five African countries. PLoS One 2020; 15(1): e0220274. doi: 10.1371/journal.pone.0220274

96. Mangesho PE, Caudell MA, Mwakapeje ER, *et al.* Knowing Is Not Enough: A Mixed-Methods Study of Antimicrobial Resistance Knowledge, Attitudes, and Practises Among Maasai Pastoralists. Front Vet Sci 2021; 8: 645851. doi: 10.3389/fvets.2021.645851.

97. Haenssgen MJ, Charoenboon N, Zanello G, *et al*. Antibiotic knowledge, attitudes and practices: new insights from cross-sectional rural health behaviour surveys in low-income and middle-income South-East Asia. BMJ Open 2019; 9(8): e028224. doi: 10.1136/bmjopen-2018-028224.

98. Velazquez-Meza ME, Galarde-López M, Carrillo-Quiróz B, *et al*. Antimicrobial resistance: One Health approach. Vet World. 2022 Mar 28;743–9. doi: 10.14202/vetworld.2022.743-749

99. Mann A, Nehra K, Rana JS, *et al*. Antibiotic resistance in agriculture: Perspectives on upcoming strategies to overcome upsurge in resistance. Curr Res Microb Sci 2021; 2: 100030. doi: 10.1016/j.crmicr.2021.100030.

100. Mdegela RH, Mwakapeje ER, Rubegwa B, *et al*. Antimicrobial Use, Residues, Resistance and Governance in the Food and Agriculture Sectors, Tanzania. Antibiotics 2021; 10(4): 454. doi: 10.3390/antibiotics10040454