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TARGETING ESSENTIAL GENETIC PATHWAYS IN MULTI-DRUG-RESISTANT MRSA AND *A. BAUMANNII*

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ABSTRACT

Global spread of infections resistant to various medications including Methicillin-resistant Staphylococcus aureus (MRSA) and Acinetobacter baumannii are a consistent danger to health of people. Complex genetic, metabolic and regulatory schemes allow these organisms to survive under tough conditions, evade immune system of their host and endure numerous antibiotic regimens. The cell envelope plays a crucial role in ensuring bacteria remain alive since it maintains the structure of the cell intact, as well as giving it a way of contacting the host. Lipid A in the outer membrane has lipopolysaccharide in Gram-negative bacteria such as the A. baumannii-bacterium. Lipid A requires the use of the enzymes lpxA, lpxC, and lpxD, and is required to enhance the stability of the membrane and its virulence. The integrity of the wall in MRSA is determined by the presence of wall teichoic acids produced by tarO, tarS, and tarM. These acids play a role in the production of peptidoglycan and resistance of the cell wall against 3 beta-lactam. The efflux pumps are regulated by genes such as AdeRS and MarR-based systems and through which bacteria become resistant to most drugs by actively exporting antibiotics. Virulence, biofilm formation and toxin production levels in MRSA and A. baumannii are regulated through quorum sensing systems such as agr and AbaI/AbaR respectively. CRISPR to target the particular genes to eliminate resistance determinants and phages to express the CRISPR system are among the new methods to treat the disease as well as artificial intelligence in finding a new drug through a synthesis of structure-based design and multi-omics data. The effectiveness and the ability to delay resistance development can be enhanced by the combination therapy plans, which involve the multiple pathways simultaneously. Despite these advances, major issues do persist, such as high rates of mutation, receiving the drug facing barriers in Gram-negative bacteria, toxicity, and rapid adaptive resistance. The contributions of looking at bacterial structural elements, regulatory networks, metabolic pathways, and virulence mechanisms represents an important shift in perspectives of how traditional antibiotics to precision antimicrobial therapy. Integrating pioneer genetic, metabolic, and computational approaches would assist us to access multidrug resistance, reduce selective pressures and improve clinical outcomes in long-term infections by bacteria that are exceedingly difficult to sanitary and persist within the body.

Keywords: *Antimicrobial resistance, multidrug-resistance, methicillin-resistance staphylococcus aureus, Acinetobacter baumannii, CRISPR antimicrobial*

INTRODUCTION

Antimicrobial resistance (AMR) has become one of the greatest health threats to the entire population in the world. The inability to discover effective treatment of infectious diseases simultaneously with the growing resistance has led to increased cases of morbidity and mortality, the escalation of hospitalization and the high cost of treatment. Recent estimates show that drug-resistant infections million with many combinations between pathogen and drugs contribute to about 4.95 deaths in the world, and not to mention, about 1.27 million deaths directly related to AMR. The rapid introduction and dissemination of multidrug resistant (MDR) pathogens have been prompted by the extensive use and misuse of antibiotics in the field of human medicine, in veterinary medicine and farm and environmental contamination with antimicrobial residues (Niño-Vega et al., 2025). AMR has significant economic impact on health care systems besides the health effect. The rise in treatment costs is due to an increase in the length of hospital stay, use of costly last- resort antimicrobial agents and punitive measures to control infection. Moreover, the use of antibiotics can disrupt more valuable microbiota posing further issues of long-term health of the patient. These difficulties emphasize the necessity of creating novel antimicrobial agents and other forms of therapy as a way to fight off exactly the resistant pathogens (Niino-Vega et al., 2025).

Following the increased menace of antimicrobial resistance, world health organization-WHO has identified a number of bacterial microbes in need of urgent research and development of new antibiotics. These include the ESKAPE pathogens who are a group of bacteria that have been observed to not only evade the action of antimicrobial drugs but also to cause serious hospital-acquired infections as well. They are *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa* and *Enterobacter* species. These organisms pose a significant health care facility problem since they are highly virulent and flexible as well as prone to antimicrobial resistance. Specifically, WHO has categorized carbapenem-resistant *Acinetobacter baumannii* as a critical priority pathogen that underscores the critical necessity to develop new antibacterial treatments that can be used in the control of infections caused by the specified organism (Marino et al., 2024; Niño-Vega et al., 2025).

Methicillin-resistant *Staphylococcus aureus* (MRSA) and *Acinetobacter baumannii* are some of the priority pathogens which have been given much attention because of the relevant importance they have on health care related infections and because of the growing magnitude of their level of resistance to antimicrobials. *Staphylococcus aureus* has frequently occurring gram positive pathogen and causes community acquired and hospital associated infections such as infections of skin and soft tissues, pneumonia and endocarditis and blood stream infections. The initial innovation of antibiotics had an effect on the situation as it lowered the mortality rates caused by *S.aureas* infections. The development of the MRSA has posed a significant treatment problem. Strains of MRSA contain mecA gene which expresses the modified pencillin binding protein PBP2a, and makes them resistant to 8-lactam antibiotics, like penicillin, cephalosporins and carbapenems. Moreover, MRSA strains frequently exhibit resistance to several other classes of antibiotics such as aminoglycosides, macrolides, flouroquinolones and tetracyclines thus making infections very compelling (Liu et al., 2025).

In the same manner, *Acinetobacter baumannii* has also become a very problematic opportunistic pathogen in hospitals. It is commonly accredited with health care related infections especially among the ill patients in critical conditions. They are ventilator associated pneumonia, blood stream infections, wound infections and urinary tract infections. The capability of *A. baumannii* to persist on hospital surfaces, medical equipment, and environmental foci greatly helps in its persistence and spread in intensive care units (ICUs) and other care facilities (Dolma & WM, 2022; Marino et al., 2024).

Staphylococcus aureus is a gram-positive, facultative anaerobic bacterium that regularly inhabits the skin and nostrils of human beings. Development of methiciline resistant strains has

caused *S. aureus* to become one of the most clinical relevant bacterial pathogens of the global scenario (Liu et al., 2025).

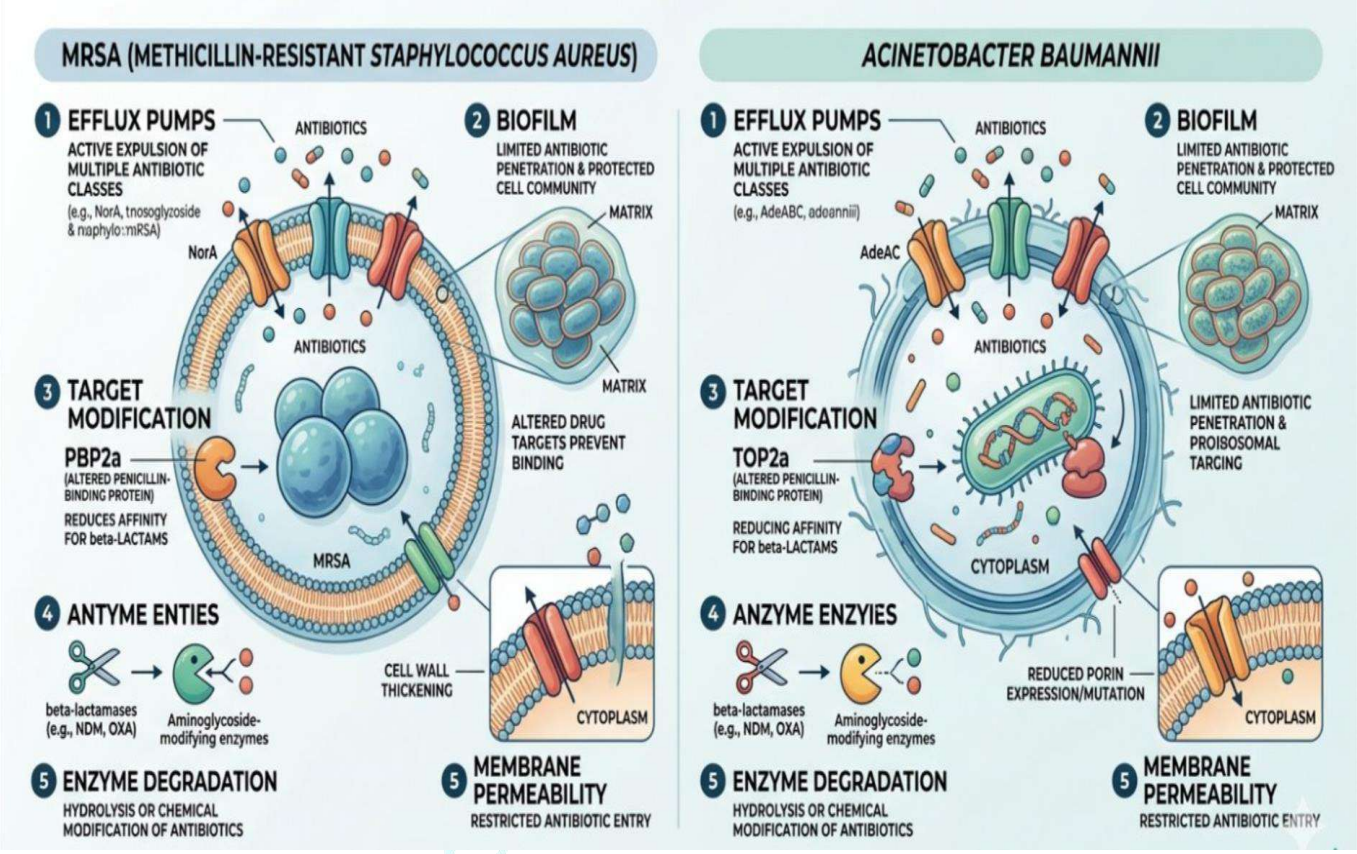

Fig 1: Mechanisms of AMR in MRSA & A. *baumanni*

Mechanism of Antimicrobial resistance MRSA

MRSA is a very tough variant of *Staphylococcus aureus* that can lead to the occurrence of trifling skin infections to the potentially lethal diseases such as sepsis, pneumonia, and blood infections. The organism has acquired resistance to many antibiotics, such as 8-lactam antibiotics through a range of genetic and metabolic activities (Nandhini et al., 2022; Liu et al., 2025). Heavily relied on the mecA gene, methicillin-resistance in MRSA is caused by it. A penicillin which has been modified. This is the gene that encodes a binding protein called PBP2a (Pencillin Binding Protein 2a). β-lactam antibiotics (methicillin and pencillin) generally prevent the development of bacterial cell walls by binding to PBPs, the cross-linking enzymes of peptidoglycan (Lade et al.,2023). Nevertheless, the protein that is generated by the mecA gene is PBP2a and is extremely lowly affined to the 1-lactam antibiotics, hence it is not capable of being bound by these drugs. MRSA can overcome antimicrobial treatment because of its ability to give rise to its cell wall even under the influence of the 2-lactamin antibiotics (Lakhundi et al., 2018).

The gene methicillin resistance is known as mecA, and it is normally encoded on a mobile genetic element known as Stapyhlococcal Cassus Chromosome mec (SCCmec) (Liu et al., 2025; Nandhini et al., 2022).

MRSA resistance through biofilm development is another mechanism that is of importance. A biofilm is an organized population of attached bacterial cells on surface which is a self-produced extra-cellular matrix made of polysaccharides, proteins, lipids and extracellular DNA. Biofilm matrix shields bacteria against environmental

stress, host immunological and antimicrobial therapy (Nandhini et al., 2022).

Efflux pumps are membrane proteins that are actively engaged in the removal of antibiotics and other dangerous substances out of bacterial cells. The efflux pumps will reduce the concentration of antibiotics in the cells of the MRSA to eliminate their entry. *Staphylococcus aureus* has been reported to have numerous efflux pumps such as NorA, NorB, NorC, MdeA, SepA, MepA, SdrM and QacA/B. These are a variety of transporter families of pumps and can export a wide variety of antibacterial chemicals. The NorA efflux pump is of special significant when it comes to the issue of fluoroquinolone antibiotic resistance. This pump will actively eliminate the fluoroquinolones when over expressed resulting in a significant reduction of antibacterial effects. Viewed as significant to the emergence of multidrug resistance in MRSA are Eflux pumps (Liu et al., 2025).

The other MRSA resistance mechanism is targeting modification in which the bacterial cell changes the molecular structure of the sites of action of antibiotics. Most antibiotics are known to attach to certain proteins or enzymes of the bacteria, which can be mutated or altered in such processes, weakening the attachment (Nandhini et al., 2022).

A good number of strains of *A. Baumannii*, however, express the carbapenemase enzyme and particularly the OXA type 0 -lactamases capable of rendering such antibiotics ineffective. These enzymes decompose the 8 rings of carbapenem antibiotics. Consequently, the synthesis of bacterial cell walls can no longer be inhibited by antibiotics derived with carbapenem. *A. baumannii* which are resistant to carbapenem become a major concern in health care facilities and can resist treatment owing to the emergence of carbapenemase positive strains (Kyriakidis et al., 2021).

The efflux pump systems produce a significant impact on the *A. baumannii* resistance to antibiotics. One of the most ubiquitous efflux pumps in *A. baumannii* is the AdeABC efflux pump that belongs to the Resistance Nodulation Division (RND) family of transporters. This is an AdeABC system constituting three components, namely, the membrane fusion protein, AdeA, the drug carrier, AdeB, and AdeC. AdeB transporter has the primary role of exporting antibiotics like carbapenems, cephalosporins, tetracyclines, and fluoroquinolone. Bacteria gain an extreme resistance to a wide variety of drugs when this efflux mechanism is overexpressed (Kyriakidis et al., 2021).

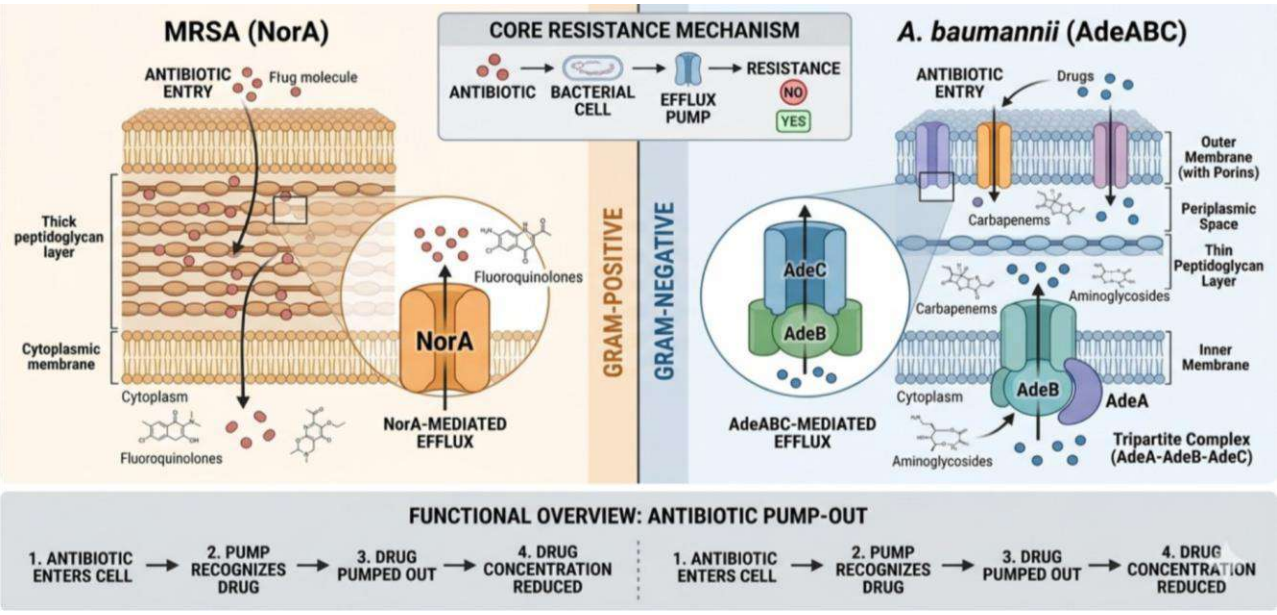

Fig 2: Efflux pump system

Alterations in the outer membrane structure also influence *A. baumannii* antibiotic resistance. CarO is a significant outer membrane protein of *A. baumannii*, which assists different compounds, including carbapenem antibiotics, to enter the cell. CarO protein is susceptible to insertions, disruptions or mutations of genes. Carbapenem resistance is resulted by decreased membrane permeability, which restricts the amount of antibiotic that is penetrated to its place of action (Kyriakidis et al., 2021; Wang et al., 2014).

The problem of horizontal gene transfer is another important factor in ensuring the spread of antibiotic resistance in bacterial populations. Resistance genes can be transferred *in A. baumannii* by integrons, transposons as well as plasmids. Having transferred these genes to another bacterial cell, it may express itself and give the cell resistance against antibiotics. The method enables the spread of resistance features among bacterial communities very rapidly, particularly in hospitals with a high level of antibiotic pressure (McNeilly et al., 2021; Kyriakidis et al., 2021).

Essential Gene Network as Novel Drug Target

Gene that is vital is the one that is needed by the bacterium in order to grow, survive and multiply. The interactions of the genes give rise to an important aspect of gene networks governing the important biological processes including DNA replication, division of cells, metabolism as well as creation of cell walls. As new avenues in the creation of antibiotics against bacteria have been realized with the formation of the bacterial genomics and molecular biology in the post genomic age (Niño-Vega et al., 2025). Of interest is the targets of these networks as therapeutic targets in combating antimicrobial resistance (AMR), these networks are able to put a strain on the normal functions of cells and lead to the death of bacteria. In addition to this, the research on the pathogens such as *Acinetobacter baumannii* that is resistant to certain antibiotics has indicated the need to investigate the cellular mechanisms that favor adaptation and survival of the bacteria during an infection (Dolma & WM, 2022).

The transposon sequencing (Tn-Seq) is a powerful technology in functional genomics; it has a potential to uncover useful genes in bacterial genomes. This method implants transposons into bacterial DNA randomly in order to avoid gene functionality. Gene that cannot receive such insertions is perforce termed as critical since it minimizes chances of survival of bacteria.

WGS assists researchers to determine potential therapeutic targets because it facilitates the identification of antibiotic resistance genes, understanding of dynamics between transmission and prediction of phenotypes of resistance. Computation and sequencing technologies have been combined to assist in the annotation of resistance genes and mobile genetic elements which are able to provide insights into the molecular processes which govern bacterial viability and antibiotic resistance (Nandhini et al., 2022). Cellular screening involves Targeting Interference with CRISPR. The advertisanced gene-silencing system called CRISPR interference (CRISPRi) comprises of a guide RNA and the catalytically inactive protein (dCas9). Unlike the traditional CRISPR-Cas9 tool, which induces the formation of a nick at both strands of the DNA image, CRISPRi prevents the activation of a specific gene by blocking the transcription of the RNA polymerase of the specific gene (Junaid et al., 2023). The two uses of CRISPR based methodologies include investigating gene functions and identification of core genes in bacterial pathogens. One of them is that the CRISPRi was effectively employed to silence genes which are important in cell replication in Acinetobacter baumannii. are advA and ftsZ, which reduced the bacteria progress significantly (Junaid et al., 2023). The given results demonstrate the feasibility of CRISPRi when looking to identify possible therapeutic target. In addition, CRISPR-Cas systems have the potential to reduce the survival rate of bacteria cells and may offer a potential therapeutic solution to address the problem of a resistant bacterial threat by targeting the genes/genes that control resistant bacteria DNA (mecA) in Staphylococcus aureus (Liu et al., 2025).

Conditionally essential genes are genes that are not essential in order to survive in a normal laboratory environment but are required when

conditions are stressful such as exposure to antibiotics, lack of adequate nutrients or pressure by the host's immune system. Modern genomic methods such as WGS and transcriptome studies can be used to research the manner in which the expression of bacterial genes responds to changing environmental conditions. These discoveries expose genes that are required during infection or antibiotic treatment that provide a new target to develop further antimicrobial agents (Niño-Vega et al., 2025).

Synthetic lethality is the genetic interaction in the sense that when two genes are disrupted simultaneously leading to cell death, disruption of all other genes has no such effect. This idea is among the priorities of antimicrobial research as it is possible to target synthetic deadly gene pairs and destroy bacterial cells. This allows the researcher to make combination medicines that are more susceptible to antibiotics and avoid resistance mechanisms by targeting genes that participate in synthetic lethal pathways (Liu et al., 2025).

The important genome mapping targets the identification of all the genes that are necessary to keep the bacteria alive in the entire genome. Whole-genome sequencing has been integrated into the list of approaches to identify antibiotic resistance genes and forecast patient bacterial resistance patterns (Niño-Vega et al., 2025).

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Two Component Regulatory Systems

The most common bacterial signal transduction pathways (TCSs) are two-component, i.e. they are systems made up of a sensor kinase located at the membrane, and a response regulator located in the cytoplasm. This is usually a linkage of the extracellular stress signals to pathways such as virulence, resistance to antibiotics, biofilm formation and cell wall homeostasis (Gotoh et al., 2010; Chen et al., 2019). TCSs have also shown the interest of antimicrobial therapy as selectively inhibited by them may disrupt critical cell survival processes but without necessarily causing cell death. This would reduce the risk of bacteria developing resistance to conventional bactericidal agents at a very short time (Gotoh et al., 2010).

It is the WalKR (synonymous YycGF or VicSR) system amongst the most crucial and stable TCSs in Gram-positive bacteria, including Staphylococcus aureus (Dubrac et al., 2008). The response regulator, which is phosphorylated to regulate the expression of downstream genes, is WalR and the signal sensor is WalK. The WalKR system directly controls enzymes of autolysins, production of peptidoglycan metabolism, and lipoteichoic acid, they are necessary to maintain the integrity of the bacterial cell wall (Dubrac et al., 2008).

The MRSA has been reported to change its resistance to cell wall-targeting drugs which involve daptomycin and vancomycin through WalKR mutations. Such changes, especially affecting cell wall thickness and turning down autolysis, could be given increased adaptive resistance under the influence of antibiotic treatment (Howden et al., 2011). Also, WalKR influences virulence features including metabolic control and biofilm formation, which implies that this TCS integrates resistance and pathogenicity control (Howden et al., 2011). This is particularly so when it comes to the creation of new agents that would go to the regulatory circuitry as opposed to a conventional metabolic or structural pathway (Dubrac et al., 2008).

The AdeRS two component system has a major role playing in the control of virulence and multidrug resistance of the Gram-negative nosocomial pathogen *Acinetobacter baumannii* by regulating AdeABC efflux pump (Coyne et al., 2010). AdeRS positively controls adaptation of adeABC transcription. The deletion of adeR or adeS reduces the expression of efflux pumps and

thus exposes an individual to aminoglycosides and other antibiotics. Conversely, AdeABC mutations or overexpression that enhance efflux activity and cause multidrug resistance in clinical isolates is often preceded by AdeRS mutations or overexpressions (Coyne et al., 2010).

Besides antibiotic resistance AdeRS has an impact on the virulence and the growth of biofilms. It has been found according to experimental studies that loss of AdeRS alters the global gene expression which reduces the formation of biofilms and reduces pathogenicity among infection models (Richmond et al., 2016).

Cell Envelope and Lipid a Biosynthesis Pathways

Lipopolysaccharide (LPS) is found in the outer membrane in Gram-negative bacteria like *A. baumannii*, and it helps to make the membrane stable and virulent. LPS lipid A component binds it to the outer membrane and serves as the main immunogenic structure signaling by host immune receptors (Ayswarya et al., 2026). Several important genes that govern the lipid A biosynthesis are lpxA, lpxC and lpxD that play a role in catalyzing early events in the biosynthetic process. The gene mutation would cause the loss of lipid A synthesis and become strains which are completely free of LPS frequently termed LPS-deficient mutants (Khan et al., 2023). Thus, bacteria strains that do not synthesize or alter lipid A, can develop resistance to antibiotics (Elanany et al., 2025). Moreover, bacteria may chemically alter lipid A in the form of modifying acyl chains or by incorporating other molecules, like phosphoethanolamine that decreases the negative charge of bacterial surface. Such alterations lower the antimicrobial peptide binding and decrease immune detection by host receptors and, as a result, increase bacterial survival in the host (Ayswarya et al., 2026).

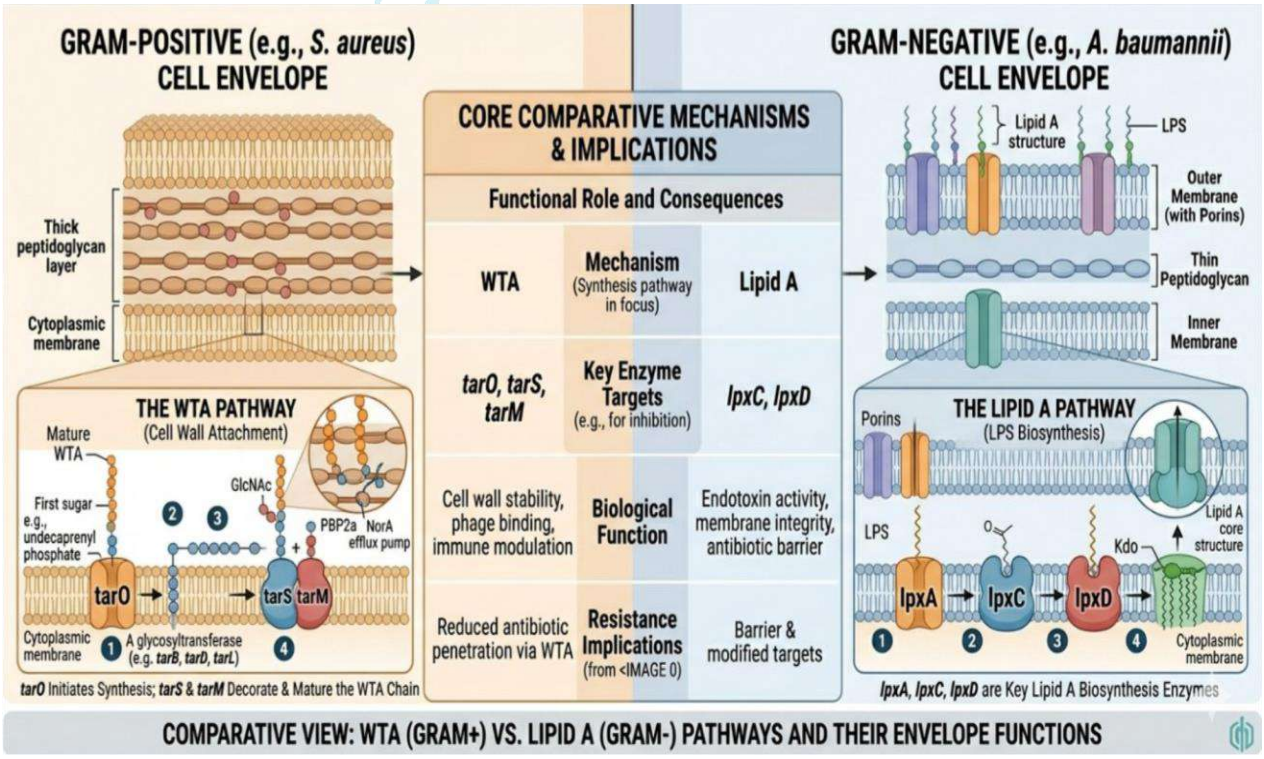

Fig 3: Cell envelope and Lipid A pathways

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a Gram-positive bacterium; methicillin-resistant bacteria stocks wall teichoic acids (WTA) within the cell envelope, which are utilized to stabilize the cell wall and regulate the mechanisms of antibiotic resistance. The WTA molecules affect the

geometrical arrangement of peptidoglycan synthesis and cell wall remodeling enzymes (Lang et al., 2024). Several genes are regulated in the production of WTA, which includes tarO, tarS, and tarM. TarO leads to the WTA biosynthetic system. The glycosyltransferases that cause glycosylation alterations of WTA are encoded by TarS and TarM (Zhou et al., 2025). Research has indicated that the tarS and tarM deletions or inhibitions inhibit the production of glycosylated WTA, which causes impairment of peptidoglycan production and weakening of the bacterial cell wall (Zhou et al., 2025). Some antimicrobial agents are demonstrated to repress the expression of tarO, tarS and tarM, which disrupt the WTA production and disrupt the cell wall integrity (Zhou et al., 2025).

A. baumannii is a hospital acquired opportunistic pathogen with the multidrug resistance. The alteration of lipopolysaccharide structures of the lipid A component is one of the major mechanisms that contributes to its persistence (Ayswarya et al., 2026). The synthesis of the lipid A is mediated by genes like lpxA, lpxC and lpxD and mutation of these genes in the pathway can result in lipid A-deficient strains with novel virulence and resistance properties (Khan et al., 2023). Besides, regulatory genes like pmrA, pmrB and eptA have a chance to modify lipid A with phosphoethanolamine group. Such a modification reduces the negative charge of the bacterial surface and leads to a reduction in binding of positively charged antimicrobial peptides, which are generated by the host immune system (Elanany et al., 2025). (Ayswarya et al., 2026).

LpxC enzyme plays an important role in the pathway of lipid A biosynthesis. Thus contributes to the central role in the synthesis of lipopolysaccharides in Gram-negative bacteria (Khan et al., 2023). Since lipid A is needed to preserve the structural integrity of the outer membrane, LpxC pathway inhibition may cause membrane formation and result in cell death of bacteria. Consequently, LpxC is found to serve as a good candidate biomolecule in the making of new antimicrobial agents to treat multidrug-resistant microorganisms like A. baumannii (Elanany et al., 2025).

BamA complex belongs to 8-ATM 218- to inserting and folding machinery of outer membrane proteins (BAM) of Gram-negative bacteria. These proteins play a vital role in keeping the membrane stable as well as interacting with the surrounding environment. OmpA is an outer membrane protein that helps to enhance bacterial virulence and membrane stability intermediating with peptidoglycan and structural stability (Oh et al., 2025).

Efflux Pump Regulatory Gene

Particular regulatory systems tend to tightly control the efflux pumps at the transcriptional stage, and disruptions of these controls can result in overexpression of the pumps as well as multidrug resistance. Thus, the expression of efflux pumps can be inhibited by aiming at regulatory genes (e.g., adeRS, regulators of the MarR type) that can potentially make bacteria susceptible to antibiotics again (Li et al., 2015; Gerson et al., 2018). The two-component regulatory system, AdeRS has been one of the most intensively examined regulatory systems, and regulates the expression of AdeABC efflux pump in A. baumannii. AdeRS has a sensor histidine kinase (AdeS) as well as a response regulator (AdeR). AdeS senses environmental cues (i.e. antibiotic exposure or cell stress) and is autophosphorylated. The phosphate is then relayed to AdeR that binds to the promoter site of the adeABC operon and induces transcription and subsequent high efflux pump production (Coyne et al., 2011; Gerson et al., 2018). The AdeABC pump is also overexpressed, which helps develop resistance to various antibiotics, such as aminoglycosides, tigecycline, and fluoroquinolones (Gerson et al., 2018).

Investigations have revealed that point mutation in either the AdeR or AdeS may produce incessant activation of the AdeABC system even without the presence of the antibiotics. These mutations increase the affinity of AdeR to DNA promoter elements that lead to continued expression of efflux pump genes and the development of increased multidrug resistance in clinical isolates

of Acinetobacter baumannii (Coyne et al., 2011; Yoon et al., 2013). Other transcriptional regulators are in control of other efflux pump systems, besides AdeRS. As an example, the AdeN controls the expression of the AdeIJK efflux pump, and the AdeL controls the AdeFGH efflux pump. Malfunctions or mutations of these regulating proteins may be a significant contributor to antimicrobial resistance by enhancing the expression of efflux pumps in bacterial pathogens (Damier-Piolle et al., 2008; Gerson et al., 2018).

The other relevant control family of efflux pumps is that of the MarR-like transcriptional regulators that are extensive across Gram-negative bacteria. The expression of marA, a transcriptional activator that is an enhancer of marA-expression, has been usually repressed by the MarR regulator in Escherichia coli, which serves to up-regulate the expression of the AcrAB-TolC efflux system. In case of mutations or environmental cues, which silence marR, marA is overexpressed inducing elevation of the efflux pump activity and multi-drug resistance (Li et al., 2015; Sharma et al., 2017).

Since these regulatory genes act inspiral to the expression of efflux pumps, they will form attractive therapeutic targets. The use of regulatory pathways as opposed to direct inhibition of the pump proteins can have the same effect of minimizing the production of the efflux pump-based antibiotics and the efficacy of the already existing antibiotics can be restored. As a result, the system of efflux pumps regulation, including AdeRS and MarR-like regulators, inhibition or modulation has become a potential approach in the creation of new-generation antimicrobial treatment (Li et al., 2015; Sharma et al., 2017).

Biofilm and Quorum Sensing Genes

Quorum sensing (QS) is a system of bacterial communication that allows bacteria to organize collective behavior depending on population density level. Bacteria in this process manufacture and secrete signaling molecules called auto inducers which accumulate into the environment. These molecules will initiate the transformation of gene expression in the bacterial population when their concentration reaches a threshold concentration (Zulfiqar & Akan, 2025).

The regulatory role quorum sensing plays in Methicillin-resistant *Staphylococcus aureus* (MRSA) is largely determined by the accessory gene regulator (agr) system that is intricate in the control of virulence and pathogenicity (Bell & Muniyan, 2025). The agr system operates based on generation of signals called autoinducing peptides (AIPs). The concentration of AIPs also rises with the increase in density of the bacteria cell. AIPs attach to AgrC receptor and activate Agr signaling cascade when reaching a certain threshold concentration (Zulfiqar & Akan, 2025).

The largest regulatory system here is RNAIII a regulatory molecule that also regulates the production of various virulence factors, and the pathogenic activity of bacteria (Zulfiqar et al., 2025). QS in A. baumannii is primarily mediated by the AbaI/AbaR regulatory system possessing control over biofilm formation and processes associated with the concept of virulence (Bell and Muniyan, 2025; Mayer et al., 2020). The system has got two key components: AbaI is an enzyme that is an autoinducer synthase that synthesizes signaling molecules called N-acyl homoserine lactones (AHLs). AbaR is a transcriptional regulator, which perceives molecules of AHL and measures the expression of the QS-associated genes. Once AHL molecules reach the critical level, they bind to AbaR which results in the expression of genes that mediate: Biofilm formation Surface motility virulence factor production. This regulatory system facilitates A. baumannii in responding to changes in the environment and helps them persist in clinical infections (Mayer et al., 2020; Bell and Muniyan, 2025).

Anti-virulence therapy is a new treatment approach where emphasis is placed on the reduction of bacterial pathogenicity, rather than causal death of bacteria. This method focuses on bacterial communication of the quorum sensing type and thus stops the expression of the virulence genes (Naderi et al., 2025). Some enzymes like lactonases and acylases break the signaling molecules prior to their association with receptors effectively interfering with QS signaling (Zulfiqar

and Akan, 2025). Anti-virulence therapy can decrease the processes of antibiotic resistance, and it could also improve the efficacy of traditional antibiotics by focusing not on bacterial survival but on virulence (Naderi et al., 2025).

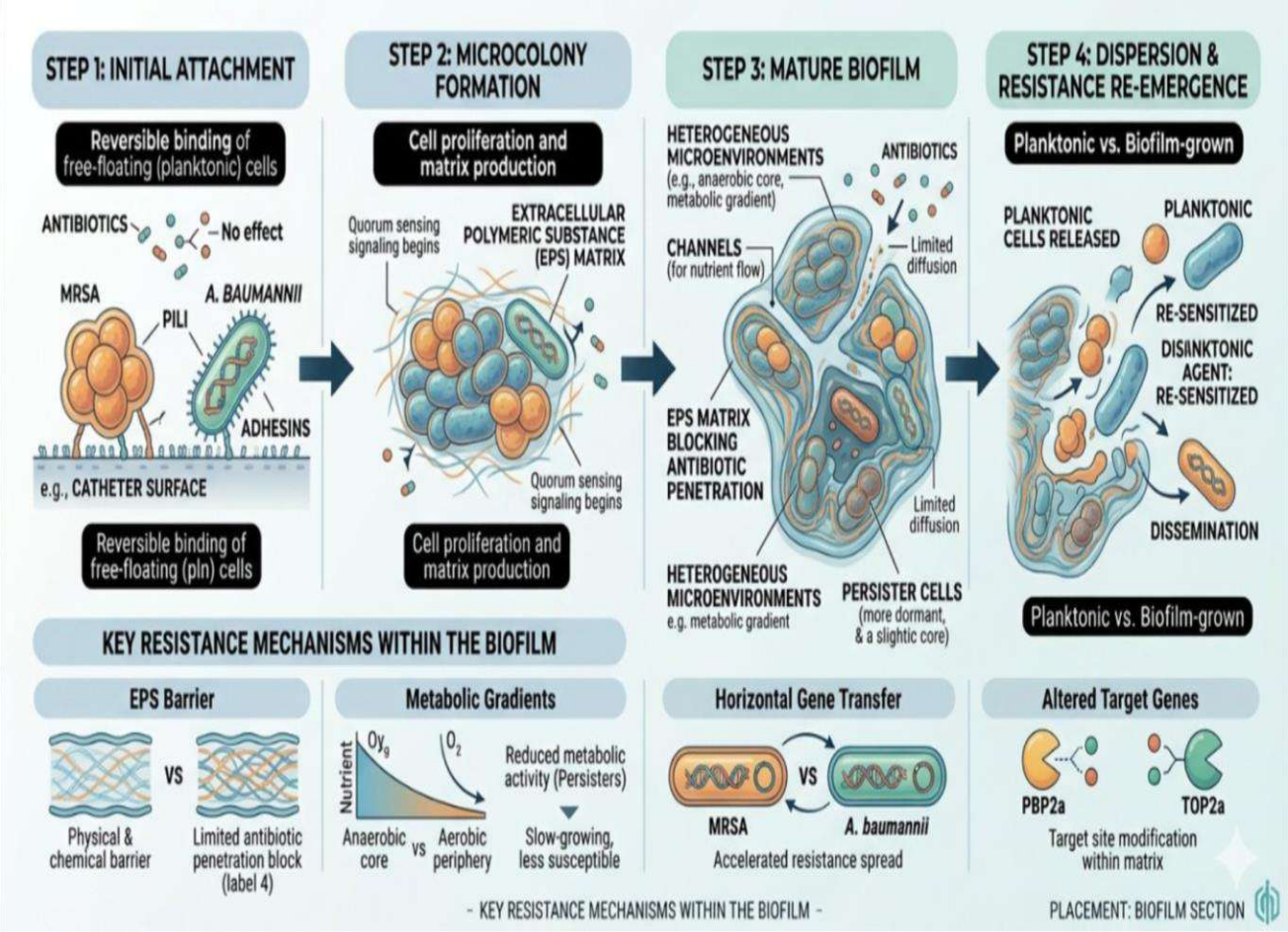

Fig: 4 Biofilm Formation and Antibiotic Resistance

CRISPR-Based Antimicrobial Strategies

Antimicrobial resistance (AMR) is increasing at a great pace, which has led to a crisis concerning the development of novel treatment plans. CRISPR-Cas technology has become an effective strategy in the fight against resistant pathogens since the tool allows bacteria DNA to be targeted in a sequence-specific manner. In contrast to traditional antibiotics that usually kill both harmful and normal bacteria, CRISPR-based antimicrobials can find the way to attack pathogenic bacteria that are resistant to antibiotics and still maintain normal microbiota. It is precisely this specificity that renders CRISPR systems excellent candidates to be used in creating precision antimicrobials or even smart antibiotics. (Ahmed et al., 2024).

CRISPR-Cas systems initially have been identified as a component of the bacterial and archaeal adaptive immune system, in which they play a role in the defense of microbial cells against invading genetic elements, including bacteriophages and bacteriophage plasmids. CRISPR mechanism occurs in three steps namely adaptation, expression and interference. In the process of adaptation, the fragments of foreign DNA are incorporated into the CRISPR array. These fragments are converted to CRISPR RNA (crRNA) in stage of expression. Lastly, during interference stage, the crRNA directs the Cas nucleases to complementary sequences which leads to cleavage of the target DNA (Ahmed et al., 2024). Due to this versatilability of targeting

sequences, CRISPR systems can be designed such that they specifically destabilize a resistance gene, or kill bacteria that are targeted with that gene. Several methods of the delivery of CRISPR parts into bacterial populations are under study, including plasmids, nanoparticles, and bacteriophages (Zuberi et al., 2024).

One of the greatest uses of the CRISPR technology in antimicrobial therapy is by targeting the elimination of antibiotic resistance genes. Within this method guide RNAs are constructed to identify unique DNA sequences that are related to the resistance determinants. During a binding event, after the CRISPR-Cas complex is bound to the target sequence, the Cas nuclease makes the DNA susceptible to antibiotics by introducing two strand-breaks in the DNA. CRISPR-Cas9 has had its success in silencing multi-resistance genes in methicillin-resistant Staphylococcus aureus (MRSA) (Ahmed et al., 2024).

In such studies, single guide RNAs (sgRNAs) were specifically designed to silence genes including mecA, aacA, grlA, and grlB which are significant in terms of antibiotic resistance. CRISPR-Cas9 plasmids expressing these genes led to the breakage of DNA and a decrease in the expression of resistance proteins, which exposed bacteria to antibiotics (Ates et al., 2024). The second benefit of CRISPR-based targeting is that it is possible to do multiplex gene editing, meaning that a number of resistance genes are simultaneously disrupted. The method is especially practical in the fight of the multidrug-resistant pathogens in which numerous resistance mechanisms may be developed in a single bacterial cell (Ahmed et al., 2024).

When it comes to inducing bacterial killing due to gene specificity, this is something that comes about in one of the biggest advantages of CRISPR-based antimicrobial systems. With the requirement that the locations pertained to by CRISPR systems are crucial genes or resistance determinants in bacteria, the DNA dual-strand breaks cause the lethal genomic damages that lead to the death of bacteria cells. Experimentally, it has been demonstrated that CRISPR-Cas9 can select antibiotic-resistant strains in a heteromixed bacterial population selectively. CRISPR targets can target the resistance of susceptible bacteria; however, CRISPR systems induce resistance in the bacterial target by impacting the gene of resistance present in the chromosome or resistance plasmid. By such a selective approach, the natural microbiota disturbance and resistance gene spread are prevented to the minimal level (Ahmed et al., 2024). Further, CRISPR technology is also promising in its activities in an effort to contain biofilm-related infections who are in most instances quite difficult to deal with the use of antibiotics. CRISPR procedures are capable of disrupting genes that are involved in biofilm formation and resistance and predispose the bacteria to the action of the antimicrobial (Zuberi et al., 2024).

One of the largest issues related to CRISPR-based antimicrobial treatment is the efficiency of the components of CRISPR into the cells of the bacteria. The most studied modes of delivery of CRISPR systems are bacteriophages (phages) that are viruses infecting bacteria. CRISPRC as components have links to phage genomes or phage mid vectors that are inserted into the phage vectors into CRISPR-delivered phage systems. When the bacterium cell is attacked by the designed bacteriophage the CRISPR machinery has been introduced to the cytoplasm. CRISPR system then finds and cuts the relevant target resistance genes or essential DNA code of bacteria that cause bacterial perishing or removal of the resistance factors (Jia et al., 2023). Nowadays, advancements in bacteriophage engineering have increased phage host range and efficacy of infection as well as permit the use of CRISPR systems to target additional bacterial pathogens. The application of engineered phages serves another purpose as an extremely powerful means of delivering CRISPR antimicrobials to groups of resistant bacteria (Jia et al., 2023). However, in spite of the tremendous claims of phage-delivered CRISPR systems there remain some shortcomings. Bacteriophages may have a low host range; i.e., each phage can only infect some bacterial strains. Furthermore, resistance to antiprotease may raise phage resistance through mutations in receptors or anti-CRISPR proteins

resulting in a reduced treatment effect (Ahmed et al., 2024).

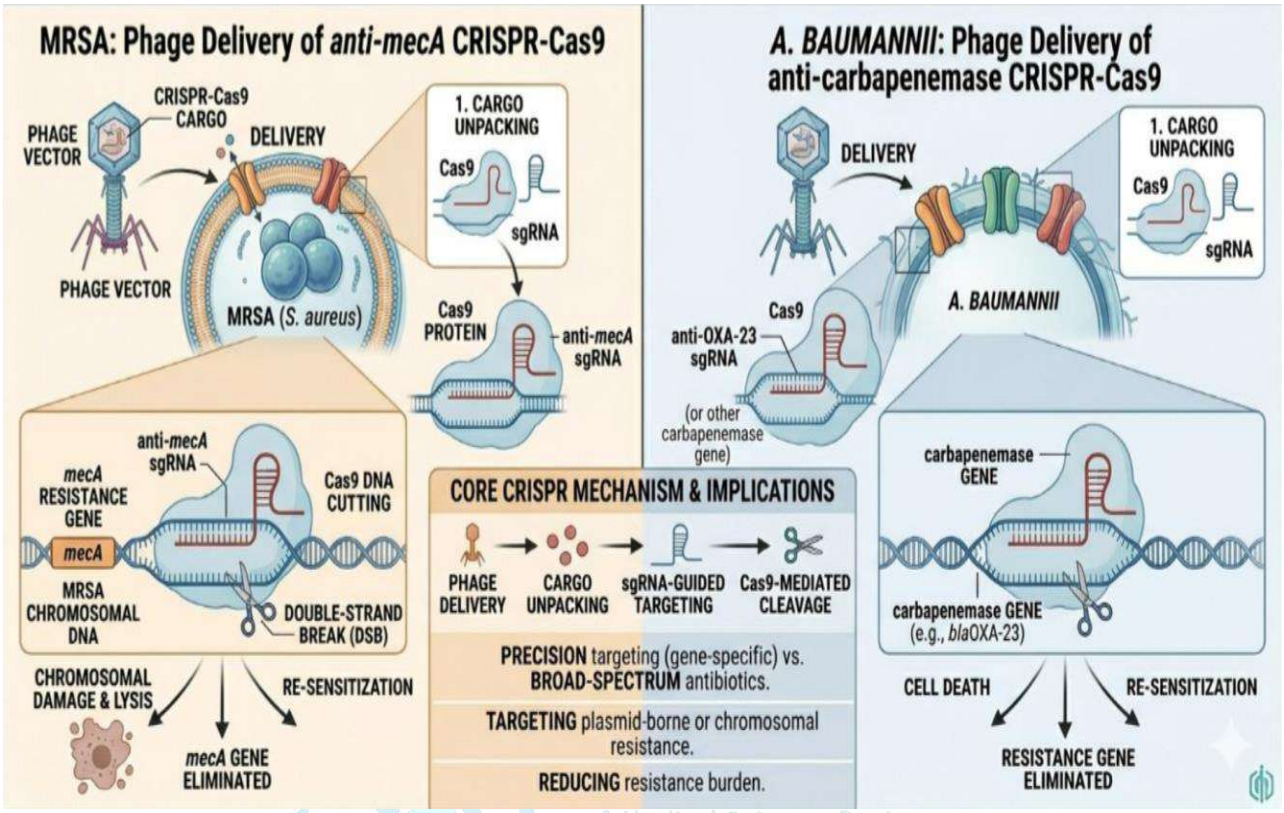

Fig: 5 How antimicrobial strategies target resistance genes

Persister Cells and Stress Response Genes

Resistant cells are composed of specialized subgroups of bacterial cells that can resist the addition of antibiotics without mutating to become genetically resistant. Those are cell types that are naturally susceptible to antibiotics but still survive due to developing a temporary dormant physiological stage altering the metabolic activities and cell development. The dormant state enables the persist cell to survive the impact of the antibiotic treatment and continue growing after removal of the stressor because most antibiotics cause the killing of actively growing cell (Vergoz et al., 2025).

The heterogeneous bacterial populations of the biofilm characterize persister cells, and have dissimilar metabolic states. Vision Biofilms facilitate protection and tolerance to antibiotics and persistence especially in pathogenic bacteria such as S. aureus and A. baumannii. Persister cells are cells that are alive even with antimicrobial treatment and might be a reservoir of recurring and chronic infections (Karimaei et al., 2021; Ma et al., 2019).

Stress response mechanisms in bacteria have a strong connection with the persistor cells. Regulatory pathways which are activated by environmental stresses such as nutrient limitation, desiccation, oxidative stress and antibiotic exposure in order to allow bacteria to adjust to the unfavorable environmental conditions. The expression of genes, accumulation of activities, and supervision of proteins in reaction to antimicrobial use is varied in response to the responses of stress (Gayoso et al., 2014; Vergoz et al., 2025). Several molecular processes that result in the formation of the persister cell involve stringent response system, toxin-antitoxin modules, metabolic regulation, and proteins degradation using proteases. These processes are in harmony, and they regulate bacterial dormancy,

stress endurance and survival in case of antibiotics exposure (Vergoz et al., 2025).

The stringent response is a regulation framework of international character that confers adaptation capability of bacteria to nutrient starvation and environmental pressures. The enzymes RelA and SpoT, involved in the production and degradation of the signalling molecules guanosine tetraphosphate and pentaphosphate, also known as (p) ppGpp, mediate the action (Pérez-Varela et al., 2020). RelA synthesizes (p) ppGpp which is an alarmone intracellular that rearranges gene expression patterns in adverse conditions like amino acid starvation. The accumulation of (p) ppGpp reduces the activities of the cell that require a lot of energy such as DNA replication, ribosomal RNA synthesis and protein expression and increases the pathways involved in survival against stress. This type of metabolism restructuring assists the bacteria cells to endure a hostile environment (Pérez-Varela et al., 2020). The phenotype of stringent response has been proven to play a role in the virulence control and antimicrobial resistance of A. baumannii. The studies have also revealed that the RelAI mutations disrupt the production of ppGpp and weaken the nature of bacteria to withstand stress these antibiotics are associated with (Pérez-Varela et al., 2020). Recent reports have also established that amino acid related metabolic pathways such as glutamate and histidine interact with the stringent response signaling to cause antibiotic resistance in A. baumannii. These pathways cause physiology and survival of bacteria when they are exposed to antibiotics (Sim et al., 2025).

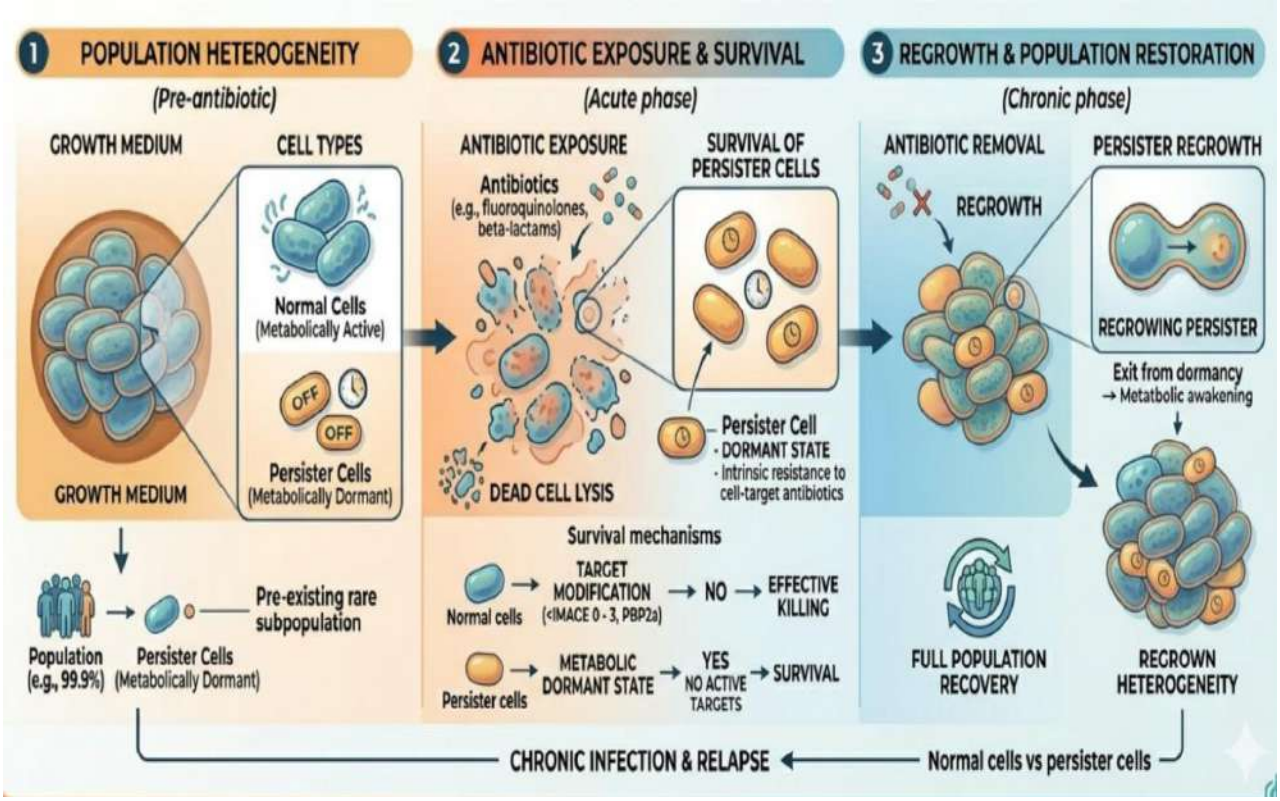

Fig: 6 Mechanism of chronic infections and regrowth Toxin-Antitoxin (TA) Systems

Small genetic modules are toxin-antitoxin systems that are common in bacterial plasmids and chromosomes. Two genes tend to encode the antitoxin protein that is unstable and the toxin protein that is stable, which deal with the modules. The antitoxin can neutralize the toxic in a healthy environment that the antitoxin will complex with the toxin and this will inhibit the toxic activity therefore allowing normal cell growth (Bahl et al., 2025). Relative to the antibiotic, either

the nutrient limitation or exposure to oxidative stress leads to the degradation of the wobbly protein complex of antitoxin in the presence of intracellular proteases under the impact of a stressful environment. The toxin progresses, instantly the antitoxin has been eliminated, which impacts key cellular aptitudes, these aptitudes encompass DNA replication, mRNA translation as well as metabolic activity. This leads to growth and development of dormant persister cells being arrested (Bahl et al., 2025). The presence of toxin-antitoxin systems is discovered to indicate considerable levels of control systems during antibiotic resistance and persistence within pathogenic bacteria. AbkAB toxin-antitoxin system is associated with tolerance and persistence in the Acinetobacter baumannii that implies that the bacteria sustain antibiotic treatment (Fernández-García et al., 2018).

Similarly, S. aureus has also been reported to have the MazEF toxin-antitoxin system that regulates biofilm formation, antibiotic resistance and acquisition of chronic infections. The stimulation of this system enhances the survival of the bacteria in case of stress and allows the long-term survival of bacteria in the tissues of the host (Ma et al., 2019). ClpP protein is significant ATP-dependent proteinases that have been utilized in the regulation of protein quality as well as regulation of stress responses in bacteria. It purifies the damaged or misfolded and unnecessary proteins that accrue in the cell in situations of cell stress to ensure homeostasis in the cell. TA systems are therefore a significant bacterial physiology as they regulate stress responses, persistence, and virulence, hence the reason as to why they are good targets to formulate new antimicrobial action (Bahl et al., 2025).

It is also in the first place of toxin- antitoxin systems controlled by ClpP on top of the quality control of the proteins. In case the body is stressed, unstable antitoxin proteins are hydrolyzed by proteins like ClpP. This makes the toxin part viable and inhibits the development of germs. The mechanism is helpful in the development of persister cells and bacterial survival in.oculated antibiotics (Karimaei et al., 2021). Articles on biofilm research on S. aureus have indicated that the toxin-antitoxin genes and the stress related proteins are up-regulated by the presence of antibiotics. It suggests that ClpP and other control systems are important in assuring that bacteria are not killed and develop resistance to antibiotics (Karimaei et al., 2021).

Moreover, there are changes in metabolism including the tricarboxylic acid (TCA) which have been associated with the process of generating persister cells in S. aureus. The disruption of the key metabolic pathways can enhance the increase in the growth of the resting persister cells and the drug resistant in bacteria (Wang et al., 2018).

Iron is a necessary micronutrient for bacteria to grow and stay alive since it is involved in numerous biological activities, such as making DNA, moving electrons, and breaking down energy. Iron, on the other hand, is securely attached to host proteins like transferrin, lactoferrin, and hemoglobin in the human body. This makes it hard for microbes to proliferate, a situation known as nutritional immunity. To get around this problem, harmful bacteria have developed several ways to get iron from their hosts. These systems are very important for bacteria's metabolism and ability to cause disease during an infection. In pathogens like *A. baumannii*, iron acquisition pathways facilitate critical metabolic processes in iron-limited environments within the host (Pérez-Varela et al., 2020). Iron is necessary for several metabolic activities, therefore not having enough of it can slow down bacterial development. Consequently, disrupting bacterial iron acquisition pathways might substantially hinder metabolic processes and pathogenicity, rendering them viable targets for antimicrobial drug development (Rocha et al., 2025).

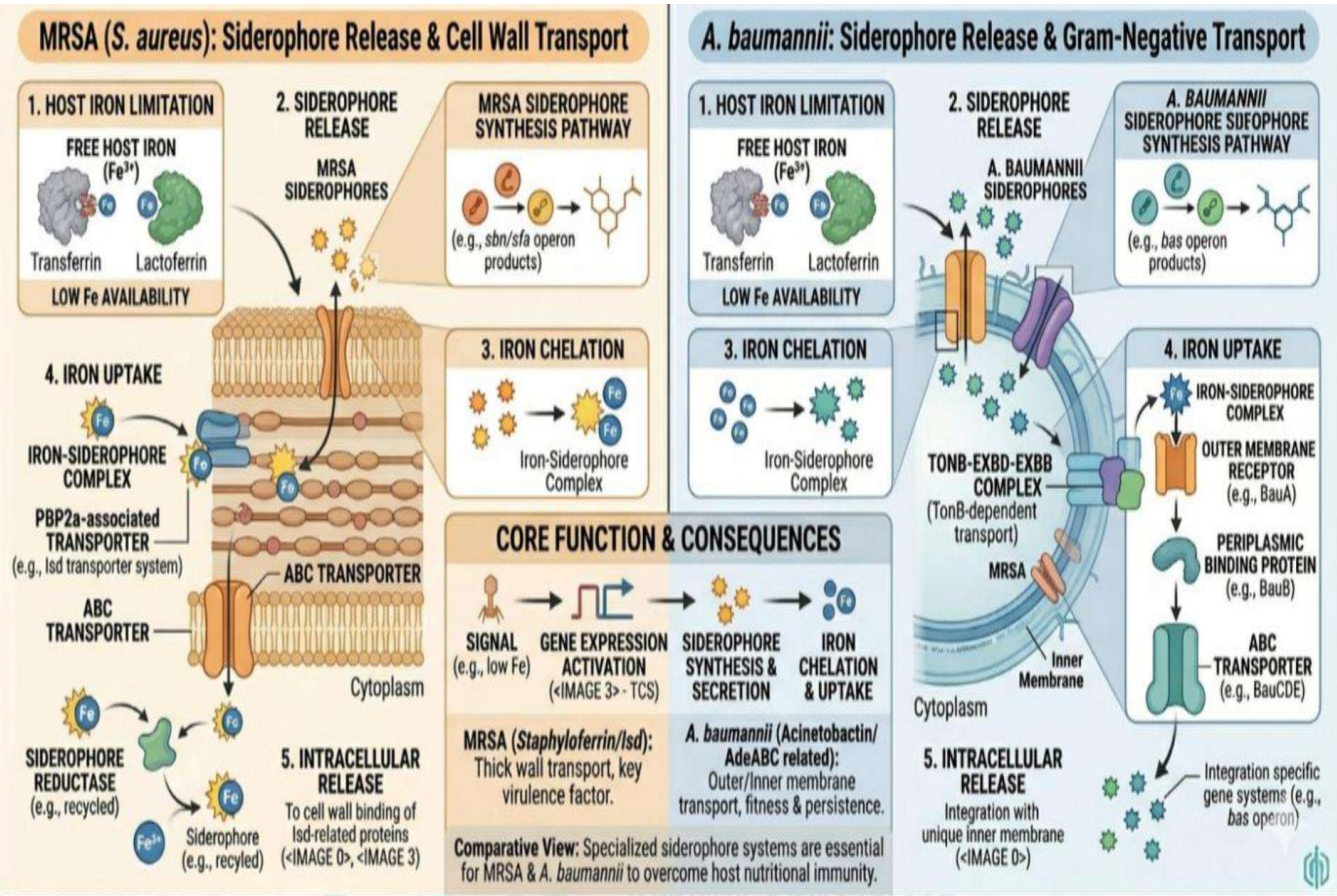

Fig 7: Iron Acquisition battle for host iron

Iron-responsive regulatory systems tightly govern how genes that are involved in taking in and using iron are expressed. The Ferric uptake regulator (Fur) protein is one of the most critical regulators. Fur is a worldwide transcriptional regulator that keeps iron levels stable in bacterial cells. Fur stops the transcription of genes that are involved in making siderophores and moving iron when there is enough iron inside the cell. But when there isn't enough iron, Fur repression stops, which turns on genes that make siderophores and suck up iron. This regulation system makes sure that bacteria have the right amount of iron for their metabolic processes without building up dangerous levels of iron (Pérez-Varela et al., 2020).

Metabolic choke points are important enzymatic events in metabolic pathways where one enzyme regulates the production or breaking down of important metabolites. Blocking these enzymes can stop an entire metabolic process and make it much harder for bacteria to survive. A lot of infections depend on metabolic pathways that are different from those of the host. For instance, enzymes that are important for central carbon metabolism, energy production, and biosynthetic pathways are necessary for bacteria to adapt and become more virulent throughout an infection (Wang et al., 2025).

Difficulties in Targeting New Genetic Pathways

Among the most significant issues in the attempt to discover new genetic pathways in creating new antibiotics, the fact that bacterial genomes evolve rapidly will be of significance. Within a short period, bacteria develop resistance to the substances of the selective pressure that drugs exert on them by altering genes, swapping genes among cells, or restructuring their genomes. Such modifications may impact the drug target or

signaling pathways, resulting in ineffective new treatments (Gadar & McCarthy, 2023).

Drug-binding or altering the protein structure is particularly likely in target-specific drugs which may develop resistance by the single point mutation in the target gene. It has been proven that mutations in genes coding significant enzymes such as DNA gyrase or topoisomerase IV render bacteria immune to fluoroquinolones. It demonstrates a rapid process of adjustment of bacteria to drugs acting on this or that genetic pathway (Silver, 2014; Nature Reviews perspective).

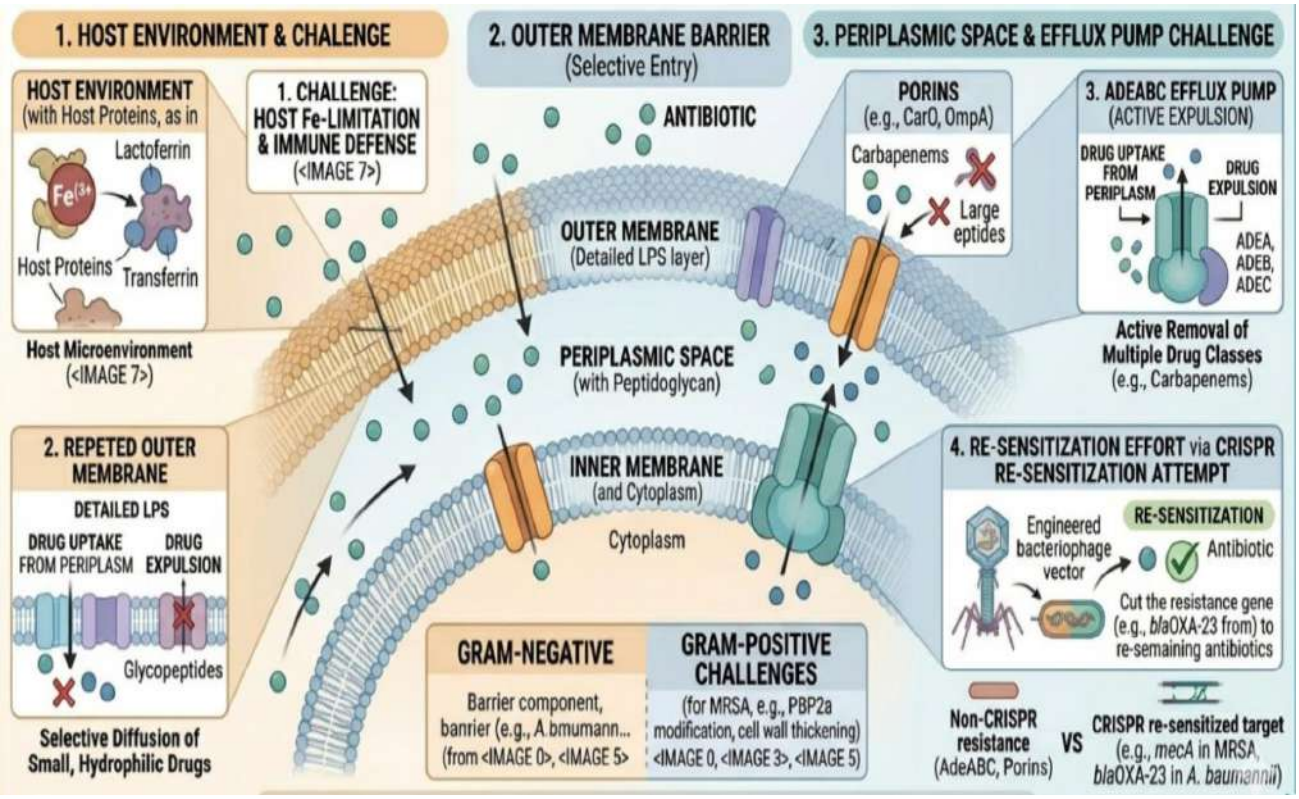

Fig 8: Key challenges for antibiotic therapy

Attacking bacterial genetic pathways, also causes people to be concerned of host toxicity and the occurrence of unexpected outcomes. Numerous bacterial metabolic and genetic pathways are partially structurally similar to those in host cells and this increases the likelihood that drugs used to control microbial targets will also affect host cell processes unintentionally. It is a particularly relevant issue when specializing in preserved processes, such as DNA replication, transcription, or translation (Gadar & McCarthy, 2023).

Though new genetic targets are discovered they are immediately beaten off by bacteria in a series of genetic processes. They are an increase in target modified by enzymes, drug degradation, increased efflux pumps and reduced membrane permeability. These could develop on their own, or they can arise in tandem, enabling organisms to circumvent the inhibitory activity of newly identified antibiotics (Nocera, 2024).

One method in modern-day drug discovery is structure-based drug design (SBDD), where biological targets are three-dimensional structures are used to design compounds with high specificity and affinity. The computer modeling, molecular docking and molecular dynamics simulations have greatly simplified drug candidate identification and optimization. To improve the prediction of ligand-protein interactions and increase binding affinity predictions, AI and machine learning methods are becoming more popular in combination with SBDD (Khan et al., 2024).

Multi-omics integration is an influential technology that can be used to understand disease etiology and to identify points of intervention in treatment, particularly through the integration of information across multiple biological levels, including genomes, transcriptomics, proteomics, metabolomics, and epigenomics. This global analysis provides researchers with complex molecular interactions that cannot be identified by single-omics methods that can provide a systems level biological understanding of disease pathogenesis (Du et al., 2024).

Conclusion

AMR is now a leading world health crisis, especially when it comes to multidrug-resistant microbes like methicillin-resistant Staphylococcus aureus (MRSA) and Acinetobacter baumannii. The traditional antibiotics are growing ineffective due to the inaccurate multidrug resistance phenotypes such as overexpression of efflux pumps, formation of biofilms, and modification of targets, horizontal gene transfer and adaptive stress response of these organisms. Consequently, there is an exigent necessity to find new therapeutic targets, rather than conventional antibiotics. Recent discoveries in bacterial genomics and molecular biology have identified a number of potential genetic and regulatory targets of bacterial survival, virulence and persistence. Cell wall biosynthesis genes (mecA, tarO, tarS, and regulatory systems; WalKR) and 2-lactam resistance genes (tarO, tarS) contribute significantly to cell wall biosynthesis and maintenance in MRSA. Likewise, lipid A biosynthesis genes (lpxA, lpxC, lpxD), and regulatory machineries like AdeRS have been found to play roles in multidrug resistance, membrane stability and virulence in A. baumannii. The efflux pump regulatory systems have become of significant therapeutic interest since they regulate the multidrug resistance pathways rather than attacking the bacterial viability itself. Similarly, biofilm formation and virulence in MRSA are regulated by the quorum sensing systems such as agr, and A. baumannii by the AbaI/AbaR systems, wherein an alternative strategy, which is anti-virulence, has lesser selective pressure to develop resistance.

Another important aspect is bacterial persistence. Persister cells can endure exposure to antibiotics by surviving via metabolic dormancy, toxin-antitoxin systems, stringent response pathways, and the presence of stress-associated proteases including ClpP. These pathways go hand in hand with persistent and repeated infections and are the future direction of antimicrobial treatment. ANOVA, CAS systems, transposon sequencing, and AI-driven drug discovery have enabled the identification of key bacterial genes and pathways relevant to drugs much more efficiently than before thanks to modern technologies like CRISPR-Cas systems, whole genome sequencing, transposon sequencing and AI-assisted drug discovery. The CRISPR-based antimicrobials are especially promising as they are able to identify in resistance genes like mecA and leave useful microbiota intact. On the same note, AI-mediated computational initiatives and multi-Omics combination are accelerating the identification of novel antimicrobial agents and combination therapy. Even with these developments, a number of problems still exist such as high rate of mutation, low drug penetration rates in Gram negative bacteria, side effects, and rapid adaptive resistance. Subsequently, development of new antimicrobial agents against them ought to take into consideration myriad targets that integrate genetic, metabolic and regulatory pathways inhibition.

Altogether, this review shows that the global trend in change of conventional bactericidal antibiotics is the shift to precision antimicrobial therapy targeting genetic and regulatory factors. Further convergence between genomics, computational biology and molecular therapeutics can offer viable strategies in the long term to overcome AMR in clinically relevant pathogens like MRSA and Acinetobacter baumannii.

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