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Breaking the Code of Staphylococcus aureus: An Expedition into Understanding its Virulence Mechanisms and Antibiotic Resistance, and the Search for Revolutionary Strategies in Antibiotic Development

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Abstract

Staphylococcus aureus is a highly adaptable bacterium that has developed sophisticated strategies to colonize humans and cause severe diseases, with a projected death toll of 10 million by 2050. The emergence and dissemination of antibiotic-resistant strains of *S. aureus* have further complicated the situation, creating an urgent need for novel antibiotics to address this global health challenge. This review presents a comprehensive analysis of *S. aureus*' virulence mechanisms, its ability to counteract host defense mechanisms, and its antibiotic resistance. It delves into how the bacterium infiltrates the skin, proliferates within neutrophils, and effectively crosses host barriers to cause severe diseases in humans. Moreover, the article highlights recent advances in antibiotic research and current approaches in antibiotic treatment. It emphasizes the importance of identifying promising drug candidates and the recent successes in the *S. aureus*-host-antibiotic paradigm. The review also discusses the potential for future developments in antibiotic drug discovery to combat resistant *S. aureus* effectively. Furthermore, the article elucidates how *S. aureus* diagnosis can aid in treatment and describes the protective mechanisms of innate and adaptive immunity cells and antibiotics. It also presents new insights into antibiotic resistance and emphasizes the discovery of promising drug candidates, highlighting the need for continued research in this area. In conclusion, the article discusses the recent successes in the *S. aureus*-host-antibiotic paradigm and outlines new developments in the pipeline for future success in antibiotic drug discovery to combat resistant *S. aureus*. It systematically reviews current research and identifies emerging research questions in this field that need to be addressed to enhance our understanding of *S. aureus* virulence and antibiotic resistance. The review provides a comprehensive perspective on the current state of knowledge of *S. aureus* and offers insights into the future direction of research in this critical area.

Keywords: *Staphylococcus aureus*, Infectious diseases, Neutrophils, Host Defense Mechanisms, Antibiotic Resistance

Introduction

Staphylococcus aureus, a Gram-positive, golden-hued, facultative anaerobic, non-motile, and skin-colonizing coccus, is one of the most prevalent human pathogens discovered by Alexander Ogston in the late 1800s [1]. The bacterium has the unique ability to clot human blood and establish a journey involved in developing tissue abscesses. It can be transmitted through various routes, including food, air, association with hospital patients, and close relationships with different communities. *S. aureus* infections can cause severe diseases, including sepsis, pneumonia, osteomyelitis, endocarditis, soft tissue infections, pulmonary tuberculosis, urinary tract infections, toxic shock illness, septic shock, bone, and joint infections [2, 3]. Rapid exploration of *S. aureus* is crucial to prevent future outbreaks, and the identification of the bacterium in a short time increases the chances of successful treatment [4, 5]. Therefore, there is a need for highly accurate and robust technology to address this issue in microbiology. Penicillin, discovered in the 1940s, was an effective treatment for *S. aureus* infections. However, two years later, penicillin-resistant *S. aureus* strains were identified, and in 1959, the introduction of methicillin, a semi-synthetic penicillin, led to the emergence of methicillin-resistant *S. aureus* (MRSA) [6, 7]. *S. aureus* has since developed excellent protective resistance mechanisms against almost all antibiotics available, challenging the clinical field to find a promising candidate to develop targeted new therapeutics for the future. Currently, there is a fast-growing interest in using natural antimicrobials to control and treat S. aureus infections in many advanced approaches [8]. This review aims to provide a detailed overview of *S. aureus*, including its nature, types, the importance of advanced methods in diagnosis, clinical manifestations, virulence mechanisms, diseases, treatment, resistance role play against host immunity, discovery in drugs, and current and future aspects in controlling *S. aureus* infection. The review highlights the need for highly accurate and robust technology in the detection of *S. aureus* and the challenges posed by antibiotic-resistant strains of the bacterium. The use of natural antimicrobials is emerging as a promising alternative in controlling and treating *S. aureus* infections.

Materials and Methods

A systematic literature review was conducted searching the PubMed database for relevant articles published from 2000 to 2021. Search terms included "*Staphylococcus aureus*", "virulence", "antibiotic resistance", "drug discovery", "immunity", and "host defense". Only peer-reviewed articles published in English language were included. The full texts of relevant articles were obtained and reviewed to identify key studies investigating S. aureus virulence mechanisms, host defense responses, and approaches to combat antibiotic resistance. Data on *S. aureus* colonization, infection mechanisms, evasion of host immunity, mechanisms of antibiotic resistance, current treatment options, novel drug candidates under development, and knowledge gaps were extracted from the studies.

Discussion

The Diversity of Staphylococci Species and Their Toxins: Implications for Disease Pathogenesis

Staphylococci species are known to cause a range of dangerous diseases. The genus *Staphylococcus* comprises seven distinct groups, including coagulase-positive species (CPS), which include *S. aureus ssp. aureus* found in humans and animals, *S. aureus ssp. anaerobius* found in sheep, S. intermedius found in dogs, horses, minks, and pigeons, *S. pseudintermedius* found in dogs and cats, *S. delphini* found in dolphins, *S. schleiferi ssp*. coagulans found in dogs' external ear, and *S. lutrae* found in otters [9]. Upon entering the host, these bacteria secrete toxins that strongly act on the cell membrane, destabilizing and damaging the host plasma membrane.

Innovative Approaches for the Rapid Identification and Characterization of *S. aureus*: A Critical Review of Traditional and Advanced Methods

There are numerous identification and characterization techniques available for *S. aureus*. Infection can occur through food contamination, person-to-person contact, hospital association, air, and contact with domestic animals, which can act as potential mediators for the transfer of *S. aureus* [10]. Without proper diagnosis and treatment, *S. aureus* can cause severe disease, and quality assurance systems such as Hazard Analysis Critical Control Point (HACCP) provide epidemiological data for infectious diseases [11]. Traditional methods rely on culturing microbes on media and characterization by biochemical tests, which are time-consuming, labor-intensive, less sensitive, and less robust [12]. These criteria have resulted in a loss of interest in the industry for regular use due to the complex need for more manpower, time consumption, and expensive procedures. Currently, several advanced methods are available to identify *S. aureus* in a shorter time frame, with robustness, high sensitivity, and reproducibility by using immunology, nucleic acid, biosensor, and nanotechnology approaches [13] as shown in fig.1. The choice of method depends on its applicability and high discrimination power. The burden of *S. aureus* can be reduced by diagnosing the target of interest to prescribe an appropriate treatment or avoid further complications. Recently, there has been an interest in identifying techniques suitable for point-of-care (POC) applications [14]. The application of a particular method depends on its nature, including cost, sensitivity, robustness, and flexibility to the process sample of interest as in table1 [10].

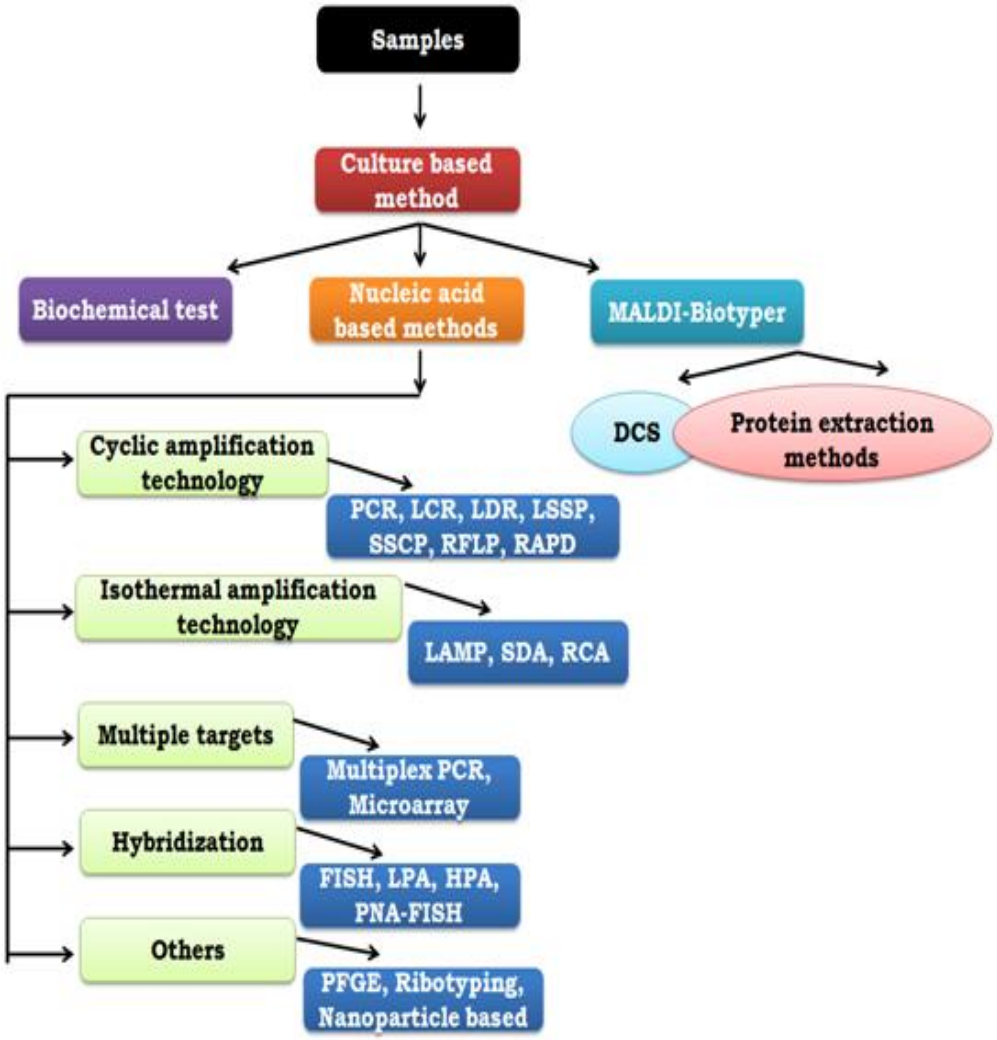


Figure 1: Schematic representation of different methods available for detection of diverse *S. aureus.*

Table 1: A overview of different parameters to be considered about few diagnostic methods in pathogen identification.

	Standard culture	**PCR**	**Sequencing**	**PCR-SSCP**	**MALDI-Biotyper**	**LAMP**
Assay time (sample processing to detection)	2-7 days	> 3 hr	Within a day	< 5 hr	< 10-30 min	<2
Instrument	Incubator, Biosafety Laminar hood Rs. 50,000-2,00,000	Standard PCR machine Rs. 3,00,000 – 4,00,000	Automated sequencer Rs. 7,00,000	Electrophoresis unit Rs.25,000	Bruker Daltonics MALDI-TOF-MS Rs. 1,20,00,000	Water bath Rs. 10,000
Cost (per assay)	~ Rs. 50 (media, plates, chemicals)	~ Rs. 60 (polymerases, dyes, primers, agarose)	~ Rs. 500 (PCR, purification, chemicals)	~ Rs. 90 (chemicals)	~ Rs. 2,500 (chemicals and instrumentation)	~ Rs. 25 (primers, dyes and enzyme)
POC use	Difficult to apply	Easily applicable	Difficult to use	Easily applicable in the future	Might be applicable in the future, currently using in facilitated diagnostic labs	Versatile for many applications
Care	Given at highest priority	Maintain hygiene	Maintain hygiene	Care should be taken	Work with sterile condition	Work with sterile condition
Sensitivity	Difficult to obtain	High	High	Moderate	Very high	Very high
Reproducibility	Rare	Depends on sample	Depends on sample	Depends on condition	Very high	Depends on sample
Disadvantage	Time consuming	High price thermal cycler and required trained person	Need trained person, powerful interpretation skill, expensive and not suitable for routine use	Need to maintain required condition and not applicable for some POC applications	High cost of initial equipment cost	Developed only for small organisms

Revolutionizing Microbial Diagnosis: The Role of MALDI-TOF MS and MALDI-Biotyper in Antimicrobial Stewardship Programs

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) is a highly precise analytical technique used to identify or quantify several compounds by measuring their peak mass/charge (m/z) ratio. Recently, its utility has expanded and it is now routinely used by many clinical microbiology laboratories in various countries [14-18]. MALDI-Biotyper, on the other hand, is specifically used to identify microorganisms present in a given sample, providing results within two minutes of gap from the isolated colonies. This can be done through two methods: direct colony smear (DCS) and protein extraction by formic acid method [19, 20]. The mechanism of MALDI-Biotyper involves preparing the sample, either DCS or protein, and placing it onto the MALDI 96 plate target, followed by repeated drying of the sample. After adding a matrix containing a-cyano-4-hydroxycinnamic acid and drying the plate, it is placed in a MALDI chamber. When the laser beam hits the matrix, the analyte-containing matrix absorbs the light and transfers it to ionize the sample. The ionized ions in the gaseous phase accelerate and travel in a magnetic field to the electrical chamber. A spectrum is then created based on the m/z ratio of ions and ion time-of-flight (TOF) taken to reach the detector, which is then decoded to give the respective spectra. The matched results arescored in the range of 1.699 to 3.000, with a red color indicating no consistency (neither genus nor species), yellow indicating genus consistency (score 1.700-1.999), green indicating probable species identification (score 2.000-2.299), and highly probable species identification by scoring a value of 2.300-3.000 [21-23] (Fig. 2).

This method is useful in giving clinicians useful data during bloodstream infections (BSIs) to help treat them with antimicrobial therapy. Studies suggest that antimicrobial stewardship (AMS) programs, which provide real-time information to improve patient health, are more effective compared to results alone [24-26]. A recent meta-analysis evaluated 31 investigations and 5,920 patients for molecular rapid diagnostic testing (mRDT) and MALDI-TOF MS on clinical outcomes in patients with BSI. The results showed that the mortality risk was drastically lower with mRDT compared to the conventional microbiology approach [27].

In this regard, we believe that mRDT is now part of the standard of care in patients with BSI and that MALDI-TOF and/or molecular-based methods for microbial diagnosis with AMS will improve clinical outcomes and reduce healthcare costs.

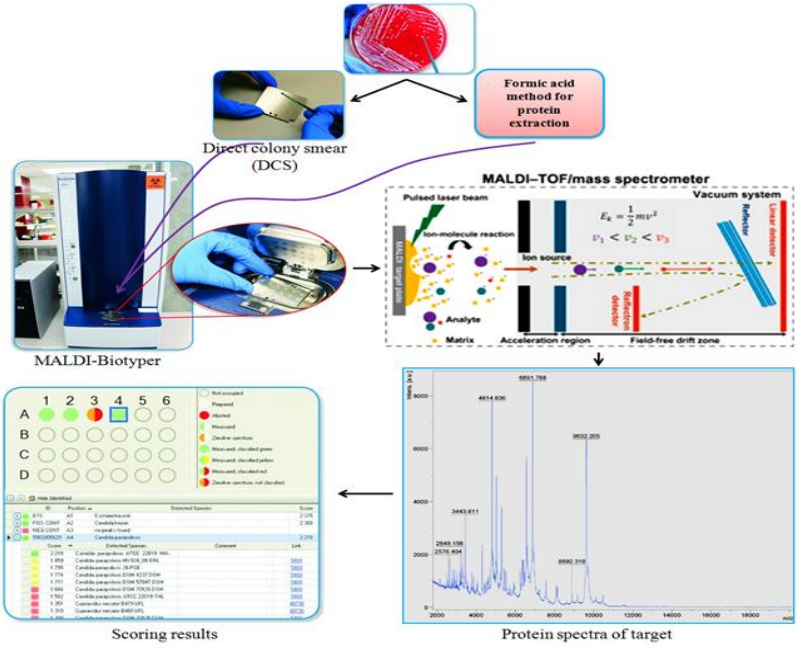


Figure 2: The principle mechanism of MALDI-TOF MS in identification of *Staphylococcus aureus*.

Utilizing MALDI-TOF MS for Assessing Antibiotic Resistance and Virulence Factors of *S. aureus:* Feasibility for Diagnosing MRSA Lineages and Rapid Antimicrobial Susceptibility Assessment

Recent studies have confirmed the effectiveness of MALDI-TOF MS for assessing antibiotic resistance strains of *S. aureus* mediated by β-lactamases (27, 28). However, if the resistance is not mediated by β-lactamases, such as through mutated porins or upregulation of efflux pumps, the method may not be suitable (29-32). In addition to antibiotic resistance, MALDI-TOF MS has also been utilized to analyze the virulence factors and resistant determinants of *S. aureus*. Several reports have highlighted the feasibility of using MALDI-TOF MS to discriminate MRSA lineages based on their fingerprints (29-32). This suggests the potential for MALDI-TOF MS to be used as a diagnostic tool for MRSA infections. Furthermore, MALDI-TOF MS has been suggested as a rapid method for antimicrobial susceptibility assessment in the future (33, 34) as in table 2. This can greatly benefit clinical practices by providing faster and more accurate results for the selection of appropriate antibiotics to treat infections caused by *S. aureus*.

Table 2: Examples of studies utilizing MALDI-TOF MS for analyzing *S. aureus* virulence factors and antibiotic resistance

Reference	Aim	Findings
27	To assess antibiotic resistance in Acinetobacter baumannii complex	MALDI-TOF MS was effective in detecting aminoglycoside resistance.
28	To identify urinary tract pathogens and their susceptibility to antibiotics	MALDI-TOF MS combined with a modified EUCAST disk diffusion test provided rapid identification and susceptibility testing of urinary tract pathogens.
29	To discriminate MRSA lineages	MALDI-TOF MS fingerprints were able to differentiate MRSA lineages, suggesting the feasibility of using this method as a diagnostic tool for MRSA infections.
30	To identify Salmonella enterica serovar Typhimurium	MALDI-TOF MS was able to determine SPI-1-controlled gene expression patterns for identifying Salmonella enterica serovar Typhimurium.
31	To develop patient-specific metabolic networks for breast cancer research	MALDI-TOF MS was used to identify the metabolic profiles of breast cancer cells for the development of patient-specific metabolic networks.
32	To identify S. aureus and detect methicillin resistance	MALDI-TOF MS was effective in identifying S. aureus and detecting methicillin resistance.

33	To identify various microorganisms	MALDI-TOF MS was able to identify bacteria, mycobacteria, yeasts, Aspergillus spp., and positive blood cultures.
34	To identify bacteria and Candida species in positive blood culture broths	MALDI-TOF MS was able to provide real-time identification of bacteria and Candida species inpositive blood culture broths, facilitating faster and more accurate diagnosis.

Regulatory Mechanisms of S. aureus Virulence and Antibiotic Resistance

S. aureus is a commensal bacterium that colonizes approximately 30% of the human body, residing predominantly in the anterior nares, on the skin, and remarkably in the heart, bloodstream, respiratory tract, and skeletal systems, as well as in the tissue surrounding implanted devices [35]. While *S. aureus* is typically a harmless commensal, it can cause acute and chronic diseases by employing an arsenal of virulence factors, such as adhesion molecules, toxins, and secreted proteins [36]. Additionally, the bacterium has developed resistance to almost all antibiotics, becoming methicillin-resistant *S. aureus* (MRSA) through the acquisition of the mecA gene into the methicillin-susceptible *S. aureus* (MSSA) genome through horizontal gene transfer (Fig. 3) [37, 38]. Studies have revealed the expression of diverse bacterial factors, many of which are two-component systems (TCSs), trans-acting factors such as sigma factors, RNA-binding proteins, metabolite-responsive regulatory proteins, and regulatory RNAs [39]. This complex network allows *S. aureus* to modify its metabolism and synthesize host counteracting virulence factors in response to external and internal signals, as well as environmental changes. Some *S. aureus* strains are different due to genome rearrangement and the acquisition of mobile genetic elements, which are involved in the evolution of the *S. aureusgenome* in response to changing conditions [40, 41]. Among trans-acting regulators, *S. aureus* has 16 TCSs that regulate the transcription of genes and operons in response to various stimuli [42]. One of the most well-known TCS systems in *S. aureus* is the accessory gene regulator (agr) system, which senses the density of bacteria to regulate further growth (Fig. 2) [43, 44]. The agr locus comprises two different transcriptional units, RNAII and RNAIII, which are regulated by P2 and P3 promoters, respectively. RNAII encodes AgrD and AgrB, which are cell density-sensing cassettes, along with AgrA and AgrC (TCS system). Membrane protease AgrB produces and processes the internal 46-amino acid precursor peptide AgrD and continuously releases it into the extracellular environment. When the threshold concentration of AgrD reaches a particular value, AgrC autophosphorylates the intracellular histidine kinase domain, and the phosphorylated group is transferred to induce regulator AgrA, subsequently activating RNAII transcript, RNAIII, and other metabolically important genes to survive under stress or starvation conditions [45]. RNAIII is an open reading frame that encodes δ-hemolysin, a cytolytic peptide belonging to the phenol-soluble modulin (PSM) family, secretary virulence factors, and regulates cell-surface adhesion virulence determinants [46-47]. Recent studies have shown that two inhibitors, solonamide B and sarvin, can inhibit AIP and AgrA, thereby controlling the expression of virulence factors in *S. aureus* infections [48]. This has led to a focus on developing inhibitors for these types of systems, which could bring about the development of novel anti-virulence drugs for therapeutic use in the near future. The intracellular survival niche of *S. aureus* is supported by producing a high level of agr to block autophagic flux, which facilitates the hiding of bacteria in autophagosomes and protects against phagocytic killing and further dissemination [49-50]. Comparative transcriptional data has shown that either AgrA or RNAIII regulates a subset of agr target genes. AgrA activates several PSMs, and RNAIII encodes one of them, while it down-regulates carbohydrate and amino acid metabolism genes. AgrA regulates the toxins PSM peptides, which are involved in the disease life cycle of *S. aureus*. Interestingly, RNAIII controls the expression of many virulent factors, such as protein A, coagulase, fibrinogen-binding protein (SA1000),

Sbi, Hla, and some transcriptional regulatory proteins, such as Rot and MgrA (Table 3). This suggests that highly regulated circuits and quorum-sensing-dependent networks controlled by RNAIII have evolved to respond to cell density and activate virulence gene expression, initiating the infection journeyin the host (Fig. 3) [50-53]. Overall, the complex regulatory mechanisms involved in *S. aureus* virulence and antibiotic resistance are crucial to developing new treatment strategies and combating the spread of MRSA infections.

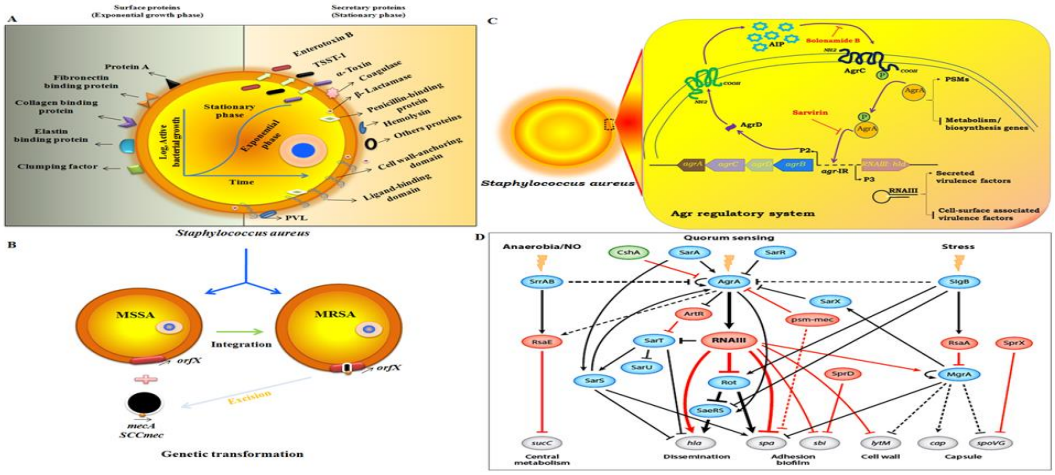


Figure 3: *Staphylococcus aureus* virulence factors and its control system. A number of virulence factors present on the surface, secreted in exponential and stationary phase during organism lifespan (A). Methicillin-susceptible *S. aureus* develops resistance to the number of synthetic antibiotics by acquiring resistance machinery *Staphylococcal* Cassette Chromosome *mec* (*SCCmec)* harbouring gene called '*mecA'* integration to become methicillin resistant (called MRSA) and vice versa (B). The *S. aureus* quorum-sensing (QS) Agr system (C) and regulatory circuits involved in virulence gene expression (D). The bold line indicates motif of RNAIII and Rot transcriptional regulator, the blue line is a transcriptional regulator, the red line is regulator RNAs, green indicates RNA helicase, gray are target mRNAs, black lines are transcriptional regulation, and red line is posttranscriptional regulation. The arrow shows activation and inhibition represents repression, and dash line indicates indirect regulation. Also, the *agr* system is controlled by many two-component systems like SarA protein families.

Table 3: Direct targets of quorum-sensing-induced RNAIII.

Gene	Protein	Functions	RNAIII-dependent regulation
Coa	Coagulase	Adhesion Fibrin clot formation	Translation repression RNase III degradation
Hla	α-hemolysin (Hla)	Pore-forming toxin Induces apoptosis	mRNA structural changes Translation activation
Hld	δ-hemolysin (Hld)	PSM toxin Hemolysis, cytolysis	Encoded by RNAIII
lytM	LytM	Cell wall metabolism Protein A release	Translation repression
mgrA	MgrA	Repressor of cell surface proteins Activator of capsule Inhibitor of autolysis	mRNA stabilization
Rot	Rot	Repressor of toxins	Translation repression RNase III cleavage
Sa1000	SA1000	Adhesion Fibrinogen-binding protein	Translation repression RNase III degradation

Sbi	Sbi	Adhesion Immune evasion Activates complement C3	Translation repression
Spa	Protein A	Adhesion Immune evasion	Translation repression RNase III degradation

S. Aureus Manipulation of Host Immune Responses in Skin Infections: Mechanisms and Implication

The normal skin activates innate immune mechanisms to prevent infections from pathogenic microbes in the environment [54]. This process is facilitated by the skin's structure, which consists of the epidermis and dermis layers (Fig. 4). The outermost layer, the epidermis, is composed of differentiated keratinocytes that are chemically cross-linked to reinforce the skin's barrier [55]. The skin's microenvironment provides protection from various factors such as pH, ultraviolet light, moisture, temperature, sebum content, and topology [54]. Sweat glands, hair follicles, and sebaceous glands significantly influence the skin's different sites. Moist sites, such as the bend of the elbow, back of the knee, and groin, are protected by sweat glands that play a critical role in thermoregulation and skin acidification, creating unfavorable conditions for specific microorganisms [56]. Sweat also contains free fatty acids, antimicrobial peptides, and other molecules that inhibit microbial colonization [57]. Sebaceous glands secrete lipid-rich sebum, which provides a hydrophobic coating and antibacterial protection to hair and skin, and they are denser in oily sites such as the face, chest, and back [54]. In response to a *S. aureus* infection, the innate immune system is activated, and it counteracts the infection using various mechanisms through its well-coordinated virulence factors (Fig. 3, Table 3) [58]. *S. aureus* can disseminate into host tissues by exiting the bloodstream, as shown in Figure 3. On the vascular endothelium surface, staphylococcal surface proteins FnBPA and FnBPB bind to fibronectin and interact with integrin α5β1, leading to cell invasion and transmigration (I). Additionally, wall teichoic acid (WTA), lipoteichoic acid (LTA), and polymers in the S. aureus bacterial envelope promote host cell invasion [59-60]. Staphylococci can induce fibrin thrombi formation through Coa/vWbp- and ClfA-mediated agglutination, binding to von Willebrand factor (vWF) on endothelial surfaces and generating Ultra Large vWF (ULVWF) polymers (II). The toxin Hla, secreted by staphylococci, binds to the ADAM10 receptor, disrupting the vascular endothelium's physiological barrier unctions [61,62] (III). Lastly, the Trojan horse model involves neutrophils with intracellular *S. aureus* extravasating and delivering bacteria into host tissues (IV) [63].

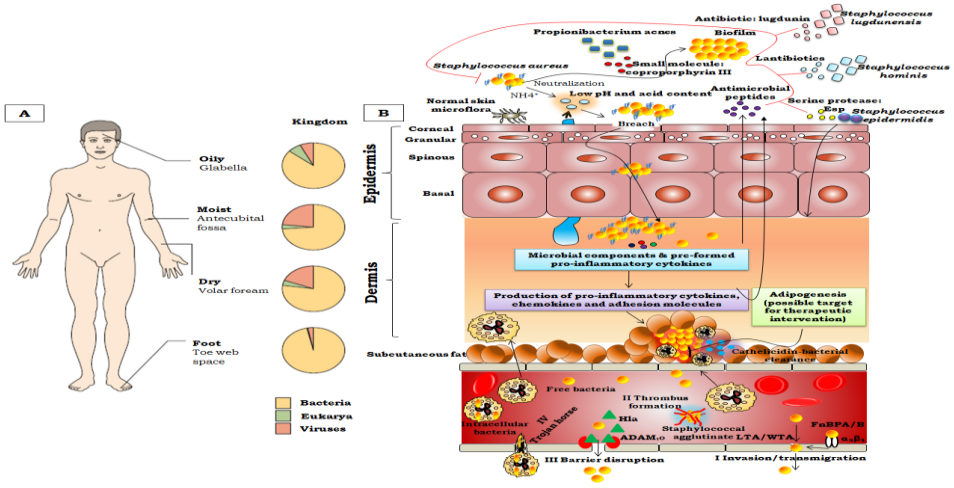


Figure 4: Skin microbial communities, skin commensal interactions and host entry of *S. aureus* with counteract/defence system. The four major microenvironments of the skin: glabella (also known as the forehead) sebaceous (oily); antecubital fossa (moist); volar forearm (dry); and toe web space (foot). The pie charts represent relative abundances of the kingdom bacteria, fungi, and

viruses across healthy adults (A). Skin microbial communities associated with many microbiotas, which can support or destroy the life of the neighbouring communities. Antibiotics produced by coagulase-negative *Staphylococcus* and specifically by *S. lugdunensis* prohibit colonization of *S. aureus*. Also, *S. epidermidis* produces serine proteases glutamyl endopeptidase (Esp) to inhibit *S. aureus* biofilm formation. Moreover, when Esp-expressing *S. epidermidis* induces keratinocytes to produce antimicrobial peptides via immune cell signalling, *S. aureus* is effectively killed. In addition, *S. hominis*-produced lantibiotics synergize with human antimicrobial peptide LL-37 to decrease *S. aureus* colonization. In contrast to inhibiting *S. aureus*, *Propionibacterium acnes* produce a small molecule, coproporphyrin III, which promotes aggregation and biofilm formation of *S. aureus*.

Staphylococcal Protein A (SpA) Manipulation of B Cell Responses in *S. aureus* Infections

S. aureus produces an immune-evasion mechanism mediated by staphylococcal protein A (SpA), which is fixed on the *S. aureus* wall envelope and released during staphylococcal growth by cell wall hydrolysis (LytM). SpA is a sortase-anchored surface protein with high affinity for vertebrate immunoglobulins such as IgA, IgD, IgG1–IgG4, IgM, and IgE. SpA has two binding capacities associated with the Fcγ and Fab domains of antibodies, and it crosslinks VH3 clan IgM of B cell receptors resulting from the binding of SpA to the Fcγ of IgG. This intern blocks staphylococcal phagocytosis and affords superantigen activity. SpA crosslinking is related to the expansion of proliferative and apoptotic collapse in B1 cells, marginal zone (MZ) B cells, and B2 cells (Fig. 5A). The overexpression of SpA suppresses the antibody response against many S. aureus antigens, and its antiphagocytic attributes provide an opportunity for the bacteria to escape and promote its journey in the blood. When these cells die, the progress of adaptive immunity is delayed during *S. aureus* infections [63-66]

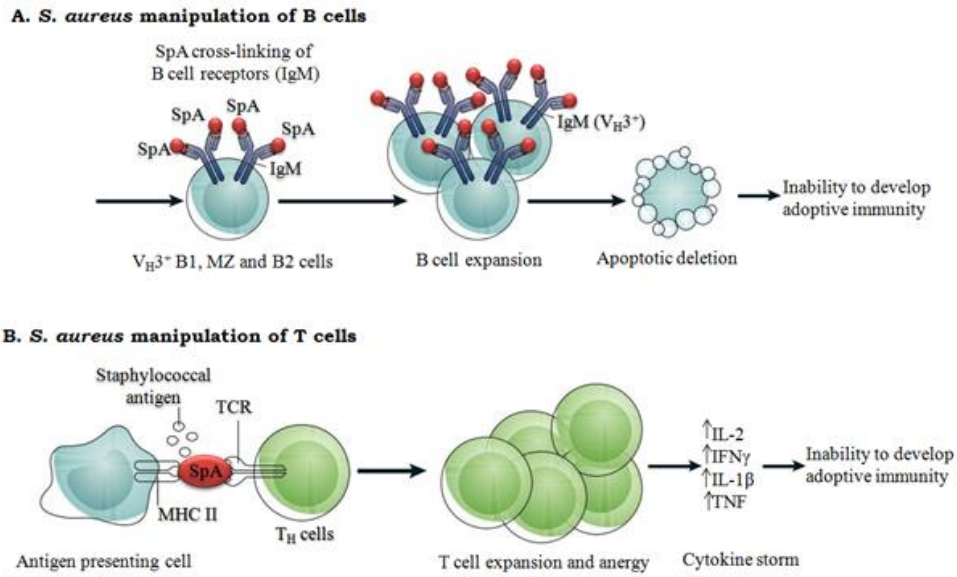


Figure 5A, B: The *S. aureus* manipulates host adoptive immunity by mediating counteracting B cell and T cell responses

Role of Enterotoxins and Superantigens in Staphylococcus aureus Pathogenesis: Manipulation of T Cell Responses and Immune Evasion

S. aureus produces twenty-three different enterotoxins and three superantigens that are associated with human diseases such as toxic shock syndrome toxin 1 (TSST1), staphylococcal enterotoxin B (SEB), and SEC. Each enterotoxin and superantigen exhibits high-affinity interactions with distinct subsets of Vβ chain T cell receptors. The secreted *S. aureus* T cell superantigen (SAg) triggers T cell

expansion and anergy and causes cytokine storms, including interleukin-2 (IL-2), interferon-γ (IFNγ), IL-1β, and tumor necrosis factor (TNF). It crosslinks major histocompatibility complex class II antigens (MHC II) on the surface of antigen-presenting cells and T cell receptors (TCRs) on the surface of T helper (TH) cells. As a result, no T cell response is elicited against *S. aureus* (Fig. 5B). S. aureus is able to manipulate and lyse T cell responses and trigger mast cell degranulation with the help of T cells lysis promotion by secreting delta toxin (Hld; also known as delta haemolysin). Hld is encrypted within the agr-regulated RNA III molecule, the regulatory arm of staphylococcal quorum-sensing [67-71].

Neutrophil-Mediated Immune Response Against Staphylococcus aureus: Mechanisms of Bacterial Clearance and Evasion

Neutrophils play a crucial role in the immune response against *S. aureus* during local infection. The recruitment of neutrophils to the site of infection is a critical step that is mediated by chemotactic stimuli. The process involves the survival of neutrophils at the site of infection and the homing of KIT+ progenitor cells, which give rise to mature neutrophils [72]. Several defects in the neutrophil and T cell responses can lead to increased susceptibility to *S. aureus*, resulting in various tissue and organ infections [73, 74]. Table 4 summarizes some of the defects that can occur in the immune response to *S. aureus*. When neutrophils encounter *S. aureus*, they use multiple mechanisms to destroy the bacteria, including phagocytosis and oxidative burst. Neutrophils generate reactive oxygen species, such as superoxide (O2–), hydrogen peroxide (H2O2), and hypochlorous acid (HOCl), to kill the bacteria. If *S. aureus* escapes into the cytoplasm of neutrophils, neutrophil calprotectin sequesters Mn2+ and Zn2+ to inhibit bacterial growth, which is known as the ‘Agr OFF’ state (Fig. 6). However, defects in neutrophil function can lead to increased susceptibility to *S. aureus* infections [75, 76]. Although *S. aureus* can be engulfed by neutrophils through phagocytosis, the bacteria can still activate the Agr quorum system to express the Agr mechanism, allowing them to escape from the neutrophils. The ROS generated during oxidative burst can inactivate the Agr-interfering peptide (AIP), which dissociates the AgrA from DNA to deactivate the Agr function, resulting in the ‘Agr ON’ state. *S. aureus* can also resist oxidative stress by producing glutathione peroxidases from the bsaA gene [77, 78]. The bacteria can then reattack the host through its toxins, such as LukAB/LukGH, LukED, PSMs, and phospholipase C, via intact Agr expression [78]. However, it is still unclear how *S. aureus* produces toxins and lyses neutrophil cells to evade the host immune system during the ‘Agr OFF’ state (Fig. 6) [78]. Further research is needed to understand the mechanisms that *S. aureus* uses to evade the immune system and to develop effective treatments for *S. aureus* infections.

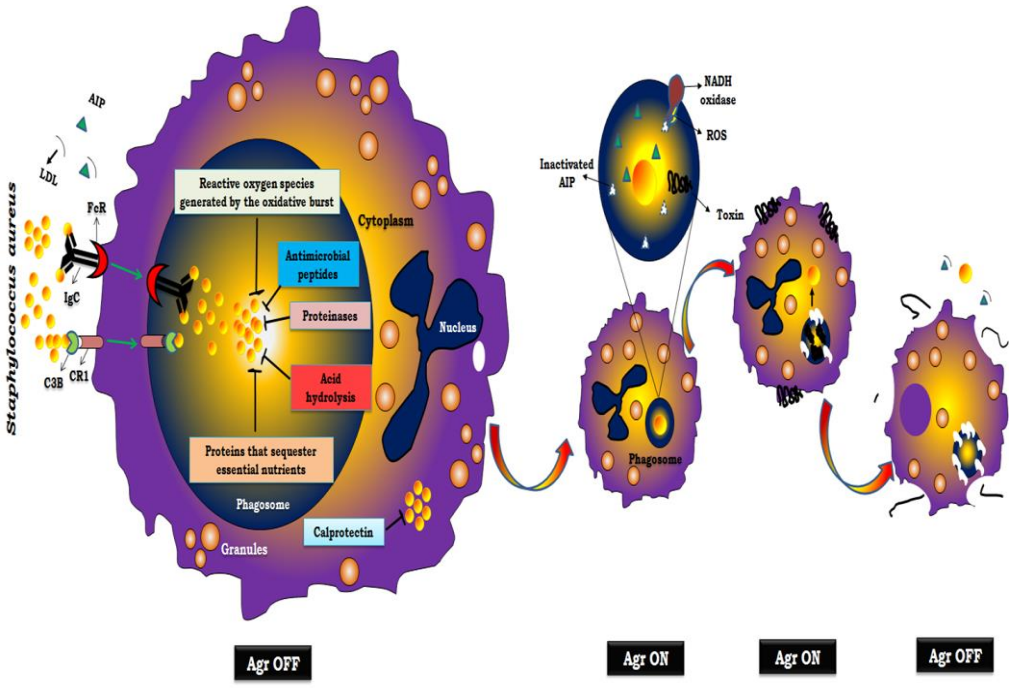


Figure 6: The neutrophil defence and *S. auerus* master counteract within neutrophil by its virulence keys.

Table 4: The *S. aureus* immune evasion determinants, their proposed function and epidemiology.

Gene	Name	Genome	Proposed Function	Target
adsA	Adenosine synthase	core	Immune suppression	Adenosine, dAdo synthesis
Aur	Aureolysin	core	Zinc protease	C3
cpsA–cpsN	Capsule	core	Phagocytosis inhibition	Not known
Chp	CHIPS	IEC1 (var)	Chemotaxis inhibition	FPR1 and C5aR
clfA	ClfA	core	Phagocytosis inhibition	γ-fibrinogen and factor I
clfB	ClfB	core	Adherence	α-fibrinogen, keratin 10 and loricrin
Cna	Cna	variable	Collagen adhesion and C1q binding	C1q
Coa	Coagulase	core	Phagocytosis inhibition	Thrombin and fibrinogen
Hld	δ-toxin	core	Mast cell activation	Not known
Eap	Eap	core	Phagocytic killing inhibition	ICAM1, C4b, elastase, cathepsin G and proteinase 3
eapH1	EapH1	core	Phagocytic killing inhibition	Elastase, cathepsin G and proteinase 3
eapH2	EapH2	core	Phagocytic killing inhibition	Elastase, cathepsin G and proteinase 3
Seb	Enterotoxin B	SaPI	T cell superantigen	Vβ TCR
Sec	Enterotoxin C	SaPI	T cell superantigen	Vβ TCR
selX	Enterotoxin like X	core	T cell superantigen	PSGL1
Ecb	Ecb	IEC2 (con)	Complement inhibition	C3d
Efb	Efb	IEC2 (var)	Complement inhibition	C3d and αMβ2 integrin
Flipr	FLIPr	IEC2 (var)	Chemotaxis inhibition	FPR2

Fliprl	FLIPrL	IEC2 (var)	Chemotaxis inhibition	FPR1 and FPR2
fnbpA	FnBPA	core	Phagocytosis inhibition and invasion	γ-fibrinogen and fibronectin
fnbpB	FnBPB	core	Invasion and adherence	α-fibrinogen and fibronectin
hlgAB	HlgAB	*sbi–hlg* (con)	Phagosome escape	CXCR1, CXCR2 and CCR2
hlgCB	HlgCB	*sbi–hlg* (con)	Phagosome escape	C5aR and C5L2
lukAB	LukAB (also known as LukGH)	*hlb–lukAB*	PMN lysis and NETosis activation	αM integrin
lukED	LukED	GIβ (var)	PMN lysis	CCR5, CXCR1 and CXCR2
lukMF	LukMF	GIβ (var)	PMN lysis	Not known
psma1	PSMα1	core	Chemotaxis and PMN lysis	FPR2
psma2	PSMα2	core	Chemotaxis and PMN lysis	FPR2
psma3	PSMα3	core	Chemotaxis and PMN lysis	FPR2
psma4	PSMα4	core	Chemotaxis and PMN lysis	FPR2
psmb1	PSMβ1	PSMb (con)	Chemotaxis and PMN lysis	FPR2
psmb2	PSMβ1	PSMb (var)	Chemotaxis and PMN lysis	FPR2
lukFS	PVL	PVL phage	PMN lysis	C5aR
Sak	Staphylokinase	IEC1 (var)	Phagocytosis inhibition	Plasminogen, fibronectin, C3 and IgG
Sbi	Sbi	*sbi–hlg* (con)	Phagocytosis inhibition	IgG Fcγ, C3 and factor H
Scn	SCIN	IEC1 (var)	Complement inhibition	C3bBb
scnB	SCIN-B	IEC2 (var)	Complement inhibition	C3bBb
scnC	SCIN-C	IEC2 (var)	Complement inhibition	C3bBb
Spa	SpA	core	Phagocytosis inhibition and B cell superantigen	Ig Fcγ and Ig Fab (V_H3)
ssl3	SSL3	GIα (var)	TLR signalling inhibition	TLR2
ssl5	SSL5	GIα (var)	Chemotaxis and platelet inhibition	PSGL1, GPCRs, GPIbα and GPVI
ssl6	SSL6	GIα (var)	Chemotaxis inhibition	PSGL1
ssl7	SSL7	GIα (var)	Phagocytosis inhibition	IgA and C5
ssl10	SSL10	GIα (var)	Phagocytosis inhibition	IgG, fibrinogen, fibronectin, thrombin and factor Xa
ssl11	SSL11	GIα (con)	Chemotaxis inhibition	PSGL1
scpA	Staphyopain	core	Chemotaxis inhibition	CXCR2
Tst	TSST1	SaPI1	T cell superantigen	Vβ2 TCR and MHC class II α-chain
Vwb	vWbp	core	Phagocytosis inhibition	Thrombin, fibrinogen, factor XIII and

Illustrates the various host-pathogen interaction strategies employed by Staphylococcus aureus during infection. The figure includes the names and abbreviations of different S. aureus virulence factors, host immune receptors, and their interactions.C5aR, C5a receptor; CCR, CC-chemokine receptor; CHIPS, chemotaxis inhibitory protein of *S. aureus*; Clf, clumping factor; con, conserved; Cna, Collagen adhesin; dAdo, deoxyadenosine; CXCR, C-X-C chemokine receptor; Eap, extracellular adherence protein; Ecb, extracellular complement-binding protein; Efb, extracellular fibrinogen-binding protein; FLIPr, formyl peptide receptor-like 1 inhibitor; FLIPrL, FLIPr-like; FPR, formyl-peptide receptor; FnBP, fibronectin-binding protein; GI, genomic island; GPCR, G protein-coupled receptor; Hlg, γ-haemolysin; ICAM1, intercellular adhesion molecule 1; IEC, immune evasion cluster; Ig, immunoglobulin; Luk, leukocidin; MHC, major histocompatibility complex; PMN, polymorphonuclear leukocyte; PSGL1, P-selectin

glycoprotein ligand 1; PSM, phenol-soluble modulin; PVL, Panton–Valentine leukocidin; SaPI, *S. aureus* pathogenicity island; Sbi, staphylococcal binder of immunoglobulin; SCIN, staphylococcal complement inhibitor; SpA, staphylococcal protein A; SSL, *S. aureus* superantigen-like; TCR, T cell receptor; TLR, Toll-like receptor; TSST1, toxic shock syndrome toxin 1; var, variable; vWbp, von Willebrand factor-binding protein.

Antibiotic Misuse and the Emergence of Multidrug-Resistant Bacterial Pathogens: Mechanisms and Implications for Public Health

The misuse and overuse of antibiotics have led to the emergence of multidrug-resistant (MDR) bacterial pathogens, posing a serious threat to public health [78-80]. Antibiotics are one of the most potent drug candidates in medicine, but their efficacy is rapidly declining due to the development of antibiotic resistance. Inappropriate use and disposal of antibiotics have resulted in the persistence of MDR bacterial strains in the environment, leading to a global potential of antibiotic-resistant determinants [81]. The history of antibiotics began about 70 years ago, with the introduction of therapeutic doses of antibiotics to kill bacteria at infected sites and clear bacterial infections without side effects in patients. The research on bacterial infections aimed to find the minimum inhibitory concentration (MIC), which is the lowest concentration of antibiotic required to inhibit the growth of target infectious bacterial populations [82]. However, the misuse of antibiotics by humans has created a worst-case scenario that has the potential to continuously transform into future generations. Antibiotics produced by bacteria and fungi are recycled in the environment, starting from the medicinal industry to the agricultural field, water resources, domestic settings, and finally into the human world on a wider scale. Humans use nearly 20%-80% of antibiotics globally, which are released directly into the environment in an active form via urine and feces [83,84]. This rational release of drugs affects bacteria in humans, animals, and plants through selective pressure (Fig. 7). This selective pressure leads to the selection and development of resistantstrains, which can transform from different environments and create a potential for global antibiotic resistance. Methicillin-resistant Staphylococcus aureus (MRSA) is a prime example of how susceptible strains can become resistant to antibiotics through multiple mechanisms (Fig. 7). These mechanisms include efflux pumps in the membrane of MRSA, alterations in the drug target that decrease or destroy the binding competence of antibiotics, master manipulator cellular enzymes that react with antibiotics to inactivate their potent function, and variations in cell permeability that hinder the uptake of antibiotics. One of the most significant mechanisms of antibiotic resistance in MRSA is the production of β-lactamase enzymes, which actively hydrolyze the β-lactam ring of antibiotics such as cephalosporins and penicillins. The efflux pumps in MRSA are proteins in the membrane that eliminate a wide variety of compounds from the periplasm to the cell, reducing the effectiveness of antibiotics. The alterations in the drug target reduce the binding competence of antibiotics and their potency to act, while variations in cell permeability hinder the entrance of antibiotics, reducing their efficacy. To combat the rising threat of antibiotic-resistant bacteria, it is crucial to develop novel antibiotics and implement effective strategies to prevent the misuse and overuse of antibiotics. Researchers are currently working to learn from the mistakes made during the antibiotic era and develop novel antibiotics to ensure infection control is balanced, and antibiotic resistance in bacteria is reduced [85].

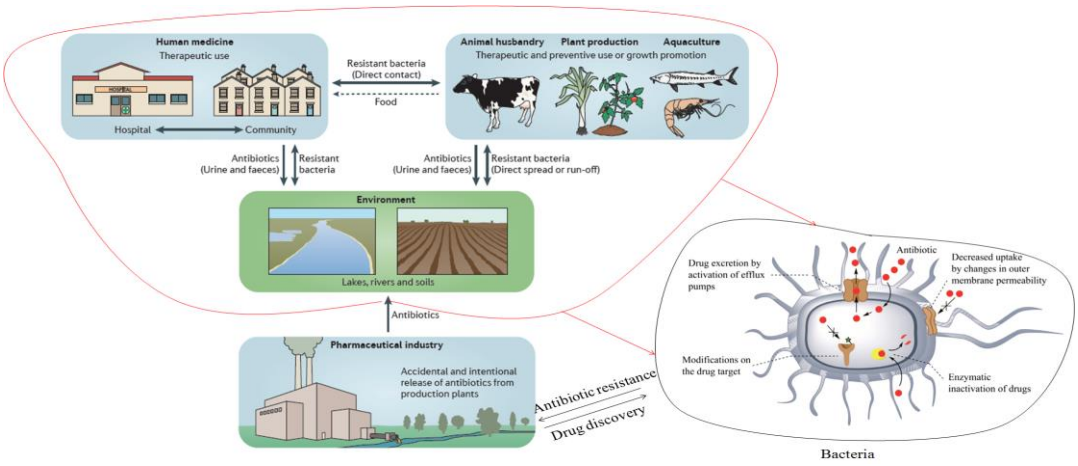


Figure 7: The returns of human antibiotics misuse was rrepresented between nature and bacteria.

Staphylococcus aureus: A Major Global Public Health Concern and a Leading Cause of Healthcare-Associated Infections and Antibiotic Resistance

S. aureus is a widespread commensal bacterium that is frequently associated with bacterial infections and has become a significant global public health concern in both developed and developing countries. It is a highly versatile pathogen, with approximately 50% to 60% of individuals intermittently or permanently colonized, making it a relatively high-risk potential for infections. Among bacterial pathogens, *S. aureus* is ranked second after Escherichia coli recovered from bacteremias in Europe in 2008 and has continuously increased its prevalence from 2002 to 2008. Recently, *S. aureus* has been reported as the second most dangerous pathogen causing healthcare-associated infections next to Clostridium difficile. In addition to its high prevalence, *S. aureus* is well-known for acquiring resistance to antibiotics [86]. The bacteria can spread from person to person by direct contact, through contaminated objects (such as gym equipment, telephones, door knobs, television remote controls, or elevator buttons), or, less often, by inhalation of infected droplets dispersed by sneezing or coughing. In the 1960s, the emergence of beta-lactam-resistant methicillin-resistant *S. aureus* (MRSA) was reported in healthcare facilities and has since become endemic, spreading worldwide in virtually all industrialized countries. MRSA infections cause a wide variety of symptoms, ranging from mild skin infections to severe life-threatening systemic infections that can beeither localized or systemic, depending on the degree of invasion and toxin production by the bacteria at the point of infection. Localized infections are commonly known as abscesses and affect the skin and soft tissues by invading bacterial pathogens, including those present in the external environment and opportunistic skin microbes [87]. *S. aureus* infections can manifest in various forms, including soft tissue infections, such as cellulitis and impetigo, as well as invasive infections, such as osteomyelitis, endocarditis, and sepsis. The clinical manifestations of *S. aureus* infections vary depending on the site of infection, the virulence of the infecting strain, and the host immune response. Infections can range from mild, self-limiting skin infections to severe, life-threatening infections that require immediate medical attention. The severity of the infections is often related to the ability of the organism to produce virulence factors, such as exotoxins and enzymes, which can cause tissue damage and facilitate bacterial spread and survival [88]. *S. aureus* is a major cause of healthcare-associated infections, including surgical site infections, bloodstream infections, and ventilator-associated pneumonia. These infections are often associated with high morbidity and mortality rates, especially in immunocompromised patients or those with underlying medical conditions. The emergence of antibiotic-resistant strains, such as MRSA, has further complicated the treatment of *S. aureus* infections and has led to increased healthcare costs and prolonged hospital stays [89]. In summary, *S. aureus* is a highly versatile pathogen that can cause a wide variety of infections, ranging from mild skin infections to severe, life-threatening systemic infections. The clinical manifestations of *S. aureus* infections depend on the site of infection, the

virulence of the infecting strain, and the host immune response. *S. aureus* is a major cause of healthcare-associated infections, and the emergence of antibiotic-resistant strains, such as MRSA, has further complicated the treatment of *S. aureus* infections. Effective management of *S. aureus* infections requires a comprehensive approach that includes infection control measures, prompt diagnosis, and appropriate antibiotic therapy.

Staphylococcus aureus Infections: From Minor Skin Infections to Life-Threatening Systemic Diseases - Understanding the Risk Factors, Clinical Manifestations, and Implications of Antibiotic Resistance

S. aureus is a pathogenic bacterium that can cause a range of minor to life-threatening diseases. *S. aureus* infections can present as abscesses on the skin or soft tissues and can spread to the bloodstream, causing bacteremia. *S. aureus* can infect almost all sites of the body, particularly heart valves, leading to endocarditis, and bones, causing osteomyelitis. The bacterium can also accumulate and spread its population throughout the body via heart pacemakers, artificial heart valves, and catheters inserted into blood vessels. *S. aureus* can cause pneumonia in the lungs, and some strains can release toxins that cause symptoms such as toxic shock syndrome, staphylococcal food poisoning, and scaled skin syndrome (Fig. 8, Table5) [90]. Several risk factors can increase the likelihood of staphylococcal infections, including leukemia, influenza, tumors, burns, surgery, diabetes mellitus, radiation therapy, chronic lung disorders, and immunosuppressive drugs such as corticosteroids. Injected illegal drugs and cancer chemotherapy agents can also suppress the immune system and increase the risk of staphylococcal infections. *S. aureus* infections can have severe consequences, including death, and are a significant cause of morbidity and mortality worldwide. The emergence of antibiotic-resistant strains, such as methicillin-resistant *S. aureus* (MRSA), has further complicated the treatment of *S. aureus*

infections, leading to increased healthcare costs and prolonged hospital stays [91]. *S. aureus* is a versatile pathogen that can cause a wide range of infections, from minor skin infections to severe life-threatening systemic infections. The clinical manifestations of *S. aureus* infections depend on the site of infection, the virulence of the infecting strain, and the host immune response. *S. aureus* is a major cause of healthcare-associated infections, and the emergence of antibiotic-resistant strains, such as MRSA, has further complicated the treatment of *S. aureus* infections. Effective management of *S. aureus* infections requires a comprehensive approach that includes infection control measures, prompt diagnosis, and appropriate antibiotic therapy. In summary, *S. aureus* infections can lead to a range of minor to life-threatening diseases, depending on the site of infection, the virulence of the infecting strain, and the host immune response. Risk factors such as immunosuppression, chronic medical conditions, and invasive medical procedures can increase the risk of staphylococcal infections. The emergence of antibiotic-resistant strains, such as MRSA, has further complicated the treatment of *S. aureus* infections. Effective management of *S. aureus* infections requires a comprehensive approach that includes infection control measures, prompt diagnosis, and appropriate antibiotic therapy.

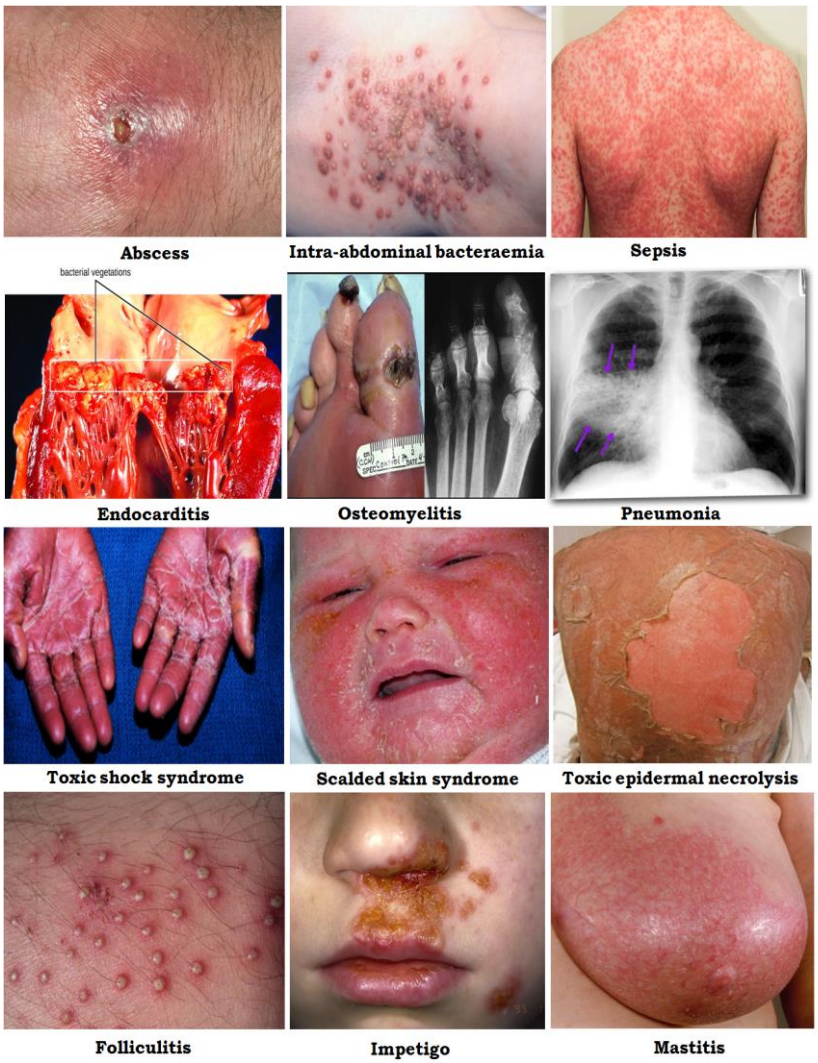


Figure 8: Different types of infections caused by notorious *S. aureus*.

Table 5: Immune Defects Associated with Staphylococcus aureus Skin Infections

Immune Defect	Diseases
Neutrophils	
Neutropenia	Severe congenital neutropenia and neutropenic patients (such as patients undergoing chemotherapy)
Defective oxidative burst	Chronic granulomatous disease, myeloperoxidase deficiency, and G6PD deficiency
Defective chemotaxis	Leukocyte adhesion deficiency type I, Wiskott–Aldrich syndrome, and RAC2 deficiency
Granule disorders	Neutrophil-specific granule deficiency and Chediak–Higashi syndrome
Combined defects in oxidative burst, chemotaxis, and phagocytosis	Diabetes mellitus and renal insufficiency (in particular, patients on hemodialysis)
Signaling	
Defects in IL-1R or TLR signaling	MYD88 deficiency and IRAK4 deficiency

T cells	
Decreased TH17 cell numbers	Hyper-IgE syndrome (caused by STAT3 and DOCK8 mutations that render patients deficient of TH17 cells), atopic dermatitis (caused by skin barrier defects, including filaggrin mutations, that lead to decreased levels of antimicrobial peptides, increased TH2 cell responses, and decreased TH17 cell responses), HIV/AIDS (which results in decreased numbers of CD4+ T cells, including TH17 cells)
IL 17F and IL 17RA deficiency (or patients with autoantibodies specific for IL 17A, IL 17F, and IL 22)	Chronic mucocutaneous candidiasis (in which patients have increased susceptibility mainly to mucocutaneous Candida infections but also to S. aureus skin infections)

This table summarizes the various human neutrophil and T cell defects associated with S. aureus skin infections. The table is divided into three columns: immune defect, which describes the specific immune system deficiency, and diseases, which lists the associated diseases. Under neutrophils, the table lists various defects that impair the function of neutrophils, including neutropenia, defective oxidative burst, defective chemotaxis, and granule disorders. Additionally, the table notes that patients with diabetes mellitus and renal insufficiency, particularly those on hemodialysis, may have combined defects in oxidative burst, chemotaxis, and phagocytosis. Under signaling, the table notes that defects in IL-1R or TLR signaling are associated with MYD88 deficiency and IRAK4 deficiency. Under T cells, the table lists various defects that affect the function of T cells, including decreased TH17 cell numbers and IL 17F and IL 17RA deficiency (or patients with autoantibodies specific for IL 17A, IL 17F, and IL 22). The table notes that these defects are associated with several diseases, such as hyper-IgE syndrome, atopic dermatitis, and chronic mucocutaneous candidiasis.

Fighting a Resilient Foe: Staphylococcus aureus Infections and the Ongoing Battle Against Antibiotic Resistance

S. aureus infections can cause a range of symptoms depending on the site of infection and the virulence of the infecting strain. Skin infections due to *S. aureus* include folliculitis, which appears as a tiny pimple at the base of a hair and is the least serious of the skin infections caused by *S. aureus*. Impetigo presents as fluid-filled blisters with honey-colored crusts that may itch or hurt. Abscesses, or furuncles, are boils with painful pus sites below the skin's surface. Cellulitis is a skin infection that spreads, causing pain and turning the skin red. Toxic epidermal necrolysis and scaled skin syndrome are serious infections that can occur in newborns and lead to large-scale peeling of the skin. All staphylococcal skin infections are contagious, and staphylococci invade the skin through wounds, follicles, or skin glands [92]. The primary defense of the host immune system against *S. aureus* infections is the recruitment of neutrophils and macrophages to the site of infection. However, the immune system can fail to defend against *S. aureus* due to the bacteria's multiple anti-defense strategies, which include sequestering host antibodies, blocking chemotaxis of leukocytes, resisting destruction after ingestion by phagocytes, or forming a biofilm to hide from the immune system. This can lead to theformation of abscesses, cellulitis, embolic infarcts, and impetigo complicating scabies infections (Fig. 8) [92]. Breast infections, or mastitis, which involve abscesses and cellulitis, can develop 1 to 4 weeks after delivery. The infection in the area around the nipple is painful and red. During breastfeeding, abscesses can release large numbers of bacteria into the mother's milk, which can then infect the nursing infant. Pneumonia caused by *S. aureus* can lead to shortness of breath, high fever, and a cough with sputum that may be tinged with blood and can turn into lung abscesses. The infection can also enlarge around the lungs, causing pleurisy and pus to collect, known as an empyema. These severe problems can make breathing even more difficult. Bloodstream infections caused by *S. aureus* are a common cause of death in people with severe burns. Symptoms typically include a persistent high fever and sometimes shock. Blood is typically a sterile environment, so the presence of live bacteria in the blood indicates an abnormal condition that can lead to severe bloodstream infections. *S. aureus* can enter the bloodstream during surgery, especially involving mucous membranes such as the gastrointestinal tract, during complications such as

pneumonia or meningitis, or due to catheters and foreign bodies that enter through arteries or veins during intravenous drug abuse. These infections can lead to severe complications such as sepsis and septic shock, which are due to the immune response to bacteria and have a high mortality rate. *S. aureus* bloodstream infections can also cause endocarditis or osteomyelitis due to the spread of bacteria through the blood into other parts of the body. To treat these infections, antibiotics or antibiotic prophylaxis can be given in high-risk situations (Fig. 8) [93-96]. Endocarditis caused by *S. aureus* infections can lead to damage to heart valves quickly, leading to difficulty breathing, heart failure, and possibly death. Osteomyelitis caused by *S. aureus* infections can cause chills, fever, and bone pain. The skin and soft tissues over the infected bone become red and swollen, and fluid may accumulate in nearby joints [92]. *S. aureus* infections can have severe consequences, including death, and are a significant cause of morbidity and mortality worldwide. The emergence of antibiotic-resistant strains, such as methicillin-resistant *S. aureus* (MRSA), has further complicated the treatment of *S. aureus* infections, leading to increased healthcare costs and prolonged hospital stays. Effective management of *S. aureus* infections requires a comprehensive approach that includes infection control measures, prompt diagnosis, and appropriate antibiotic therapy [93,94]. In summary, *S. aureus* infections can cause a range of symptoms, depending on the site of infection and the virulence of the infecting strain. Skin infections due to *S. aureus* are contagious and can lead to serious complications. Breast infections, pneumonia, bloodstream infections, endocarditis, and osteomyelitis are some of the severe complications caused by *S. aureus* infections. The immune system's failure to defend against *S. aureus* due to its anti-defense strategies can lead to the formation of abscesses, cellulitis, embolic infarcts, and impetigo. Effective management of *S. aureus* infections requires a comprehensive approach that includes infection control measures, prompt diagnosis, and appropriate antibiotic therapy. The emergence of antibiotic-resistant strains, such as MRSA, has further complicated the treatment of *S. aureus* infections and is a significant global public health concern [945-97].

Diagnosis of Staphylococcus aureus Infections: Approaches, Challenges, and Advances in the Era of Antibiotic Resistance

S. aureus infects the body in a variety of ways, leading to diverse clinical manifestations. The ability of some *S. aureus* strains to colonize the body harmlessly makes it challenging to diagnose an infection site accurately. To diagnose *S. aureus* infections, it is important to isolate the bacteria causing the infection at appropriate specimen sites, followed by identification of its toxins or measurement of its antibody in special cases such as deep-seated infections or food poisoning. Antimicrobial therapy is also crucial for understanding infection control [98]. In some cases, doctors may suspect the severity of osteomyelitis, and further diagnostic tests such as X-rays, computed tomography (CT), magnetic resonance imaging (MRI), radionuclide bone scanning, or a combination of these tests may be performed. These tests can show where the damage is and help determine the severity of the infection [98]. Prompt diagnosis and appropriate treatment are crucial in *S. aureus* infections, as the emergence of antibiotic-resistant strains has complicated treatment and increased healthcare costs. In addition, *S. aureus* infections can lead to severe complications, including death, and are a significant global public health concern [99].

In instantaneous, *S. aureus* infections can lead to diverse clinical manifestations, making it challenging to diagnose an infection site accurately. Isolation of the bacteria causing the infection at appropriate specimen sites and identification of its toxins or measurement of its antibody are crucial for prompt diagnosisand appropriate treatment. Diagnostic tests such as X-rays, CT scans, MRI, and radionuclide bone scanning may also be performed to help determine the severity of the infection. Effective management of *S. aureus* infections requires a comprehensive approach that includes infection control measures, prompt diagnosis, and appropriate antibiotic therapy. The emergence of antibiotic-resistant strains, such as methicillin-resistant *S. aureus* (MRSA), has further complicated the treatment of *S. aureus* infections and is a significant global public health concern [98-100].

Preventing Staphylococcus aureus Infections: Simple Precautions and Infection Control Measures for Combating Antibiotic Resistance

Prevention of *S. aureus* infections can be achieved through simple precautions such as washing hands with soap and water, with or without antibacterial hand sanitizers or gels. People suffering from staphylococcal infections should not handle food. Susceptible individuals should avoid cosmetic shaving on their legs and arms to prevent minor cuts and abrasions, and adequate bandages should be used to keep wounds clean, dry, and covered. Contaminated items such as clothes, towels, uniforms, equipment, and razors should not be shared to avoid transmission of the bacteria. In healthcare settings, infection control measures are crucial to prevent the transmission of *S. aureus* infections. Health care workers should practice hand hygiene before and after patient contact, use personal protective equipment such as gloves and gowns when appropriate, and follow standard precautions for the care of all patients. Patients with confirmed or suspected *S. aureus* infections should be isolated as soon as possible to prevent the spread of infection to other patients and healthcare workers [101]. Antibiotic prophylaxis may be considered in high-risk situations, such as surgery or in patients with weakened immune systems who are at high risk of developing *S. aureus* infections. However, the overuse or inappropriate use of antibiotics can lead to the emergence of antibiotic-resistant strains of *S. aureus*, such as methicillin-resistant *S. aureus* (MRSA), which is a significant global public health concern [101, 102]. In summary, preventing *S. aureus* infections requires simple precautions such as hand hygiene, avoiding sharing contaminated items, and using appropriate wound care measures. In healthcare settings, infection control measures are crucial to prevent the transmission of *S. aureus* infections. Antibiotic prophylaxis may be considered in high-risk situations, but the overuse or inappropriate use of antibiotics can lead to the emergence of antibiotic-resistant strains of *S. aureus*. Effective prevention and control measures are crucial in combating the emergence and spread of antibiotic-resistant *S. aureus* strains and reducing the burden of *S. aureus* infections worldwide [101,102].

Combatting Antibiotic Resistance in Staphylococcus aureus Infections: Challenges and Innovations

S. aureus infections remain common and serious in all public sectors, and it will continue to be a significant cause of human infections due to the emergence of resistant MRSA strains rapidly and increasing antimicrobial resistance. The number of new compounds, such as limited groups of oxazolidinones, quinolones, quinupristin-dalfopristin, various combinations, and newly investigated compounds, have been developed, but unfortunately, none of these agents has been clinically tested on a sufficient scale. β-lactam antibiotics such as dicloxacillin or flucloxacillin are typically tested against MSSA and for the treatment of superficial infections caused by *S. aureus*. Amoxicillin, co-amoxyclav, erythromycin, and topical fusidic acid or mupirocin are also used [103]. For the treatment of skin and soft tissue infections, clindamycin has been used, and vancomycin remains a first-line therapy for severe infections caused by MRSA. Other agents such as clindamycin, daptomycin, linezolid, and quinupristin-dalfopristin are used as intravenous treatments for MRSA infections [104]. The failure and confounding issue in treating *S. aureus* infections are due to several factors, including a lack of knowledge about the factors (both host and microbe) that contribute to protective immunity against *S. aureus* infections, the absence of sensitiveand robust diagnostic tools for pathogen identification to prescribe proper treatment, the emergence of antibiotic-resistant strains due to the overuse or inappropriate use of antibiotics, and the absence of proper management systems. To overcome these challenges, various strategies are being explored, such as the development of novel antibiotics, the use of combination therapies, the repurposing of existing drugs, and the use of immunotherapy and vaccines. The use of bacteriophages, which are viruses that specifically target and kill bacteria, is also being investigated as a potential treatment option for *S. aureus* infections. In addition, efforts are being made to improve the diagnosis of *S. aureus* infections through the development of rapid diagnostic tests, such as polymerase chain reaction (PCR) assays, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS), and next-generation sequencing (NGS)

technologies, which can enable accurate and rapid detection and identification of *S. aureus* strains. Effective management of *S. aureus* infections requires a comprehensive approach that includes infection prevention and control measures, prompt and accurate diagnosis, and appropriate antibiotic therapy. The emergence and spread of antibiotic-resistant *S. aureus* strains, such as MRSA, emphasize the urgent need for continued research and innovation in the development of new antibiotics, diagnostic tools, and alternative treatment options to combat this global public health challenge [103-106].

Combatting Antibiotic Resistance: Innovations and Challenges in Antibiotic Discovery

The widespread use of antibiotics to treat infections in both humans and animals has led to the emergence of antibiotic resistance, which poses a significant threat to public health. The discovery of antibiotics, such as penicillin and streptomycin, in the mid-20th century revolutionized the treatment of bacterial infections caused by gram-positive infectious agents, including Staphylococcus, Streptococcus, and Mycobacterium tuberculosis. However, the golden era of antibiotic discovery did not last long, and the emergence of antibiotic resistance in bacteria, coupled with the lack of new antibiotic discoveries, has become a major challenge in the field of infectious disease management. To develop new antibiotics, various criteria must be considered, including efficacy in treating infections with minimal toxic side effects. Antibiotics can cause alterations or destroy target bacteria in numerous ways, as discussed earlier. In the golden era of antibiotic discovery, microbial natural scaffolds were utilized to serve as antibiotic arsenals. However, the evolution of microbes and metabolites has made it difficult to identify new effective drug scaffolds. With the development of antibiotic-resistance genes in bacteria, the medicinal chemistry approach was evolved to solve the issue in an innovative way of antibiotic discovery against various pathogens to avoid resistance. The success of antibiotic discovery of new scaffolds lasted until the early 1990s when resistance became a fresh wave, leading to the emergence of innovative drug-discovery approaches in therapeutic areas using new technologies, such as manipulation of recombinant DNA to create a large libraryof desired proteins to enable rational drug design. However, in two decades, no medicine was discovered effectively. In the future, discovery programs need to focus on unconventional targets using narrow-spectrum agents, innovative models associated with a diagnosis to predict specific strategic approaches to see success in the future era. Currently, antibiotic resistance is a significant concern, and authorities such as the World Health Organization (WHO) and the Centers for Disease Control and Prevention (CDC) have reported that antibiotic resistance is a more serious threat than global warming and terrorism. The frequency of antibiotic use for treating infections caused by ESCAPE pathogens Enterococcus faecium, *S. aureus*, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter spp. is very important, but the bad news is the lack of potential alternative antibiotics. Gram-positive and Gram-negative bacteria can cause severe infections through many mechanisms, but new classes of antibiotics have not been available for more than 40 years for Gram-negatives. The antibiotic discovery process is not an easy prediction in the era of drug discovery due to evolving strains becoming resistant to existing antibiotics, making it difficult to develop novel mechanisms to overcome drug resistance. Many big pharmaceutical and biotechnology companies have left the era of drug discovery against various threats, including multiple-drug resistant bacterial strains of emerging pathogens such as MRSA and VRSA. Many factors are involved in diverting efforts, but the fact remains that investing in antibioticdiscovery against many other diseases remains a priority. Strict regulatory requirements, suitable environments, and public and shareholder expectations for the development of drug candidates are also factors that make the antibiotic discovery process challenging. The optimization of lead molecules is a tough and lengthy phase in antibacterial agent discovery, and success can be achieved through the mutual efforts of medicinal chemistry and biological science. The success percentage rate in Phase I, according to GlaxoSmithKline (GSK) metrics and clinical outcomes presented based on Centers for Medicines Research (CMR) statistics, is shown in Figure 9C. The figure illustrates the time and risk associated with drug discovery, and the success rate in Phase I is approximately 50%, which requires a committed team of scientists to work for uninterrupted 5 years to achieve. In conclusion, the emergence of antibiotic resistance poses a significant challenge to public health, and the lack of new

antibiotic discoveries is a major concern. Future antibiotic discovery programs need to focus on unconventional targets, using narrow-spectrum agents and innovative models associated with a diagnosis to predict specific strategic approaches to overcome drug resistance. Efforts to develop new antibiotics require a concerted effort between medicinal chemistry and biological sciences, and investment in such programs remains critical to combat antibiotic resistance and emerging infectious diseases [107-116].

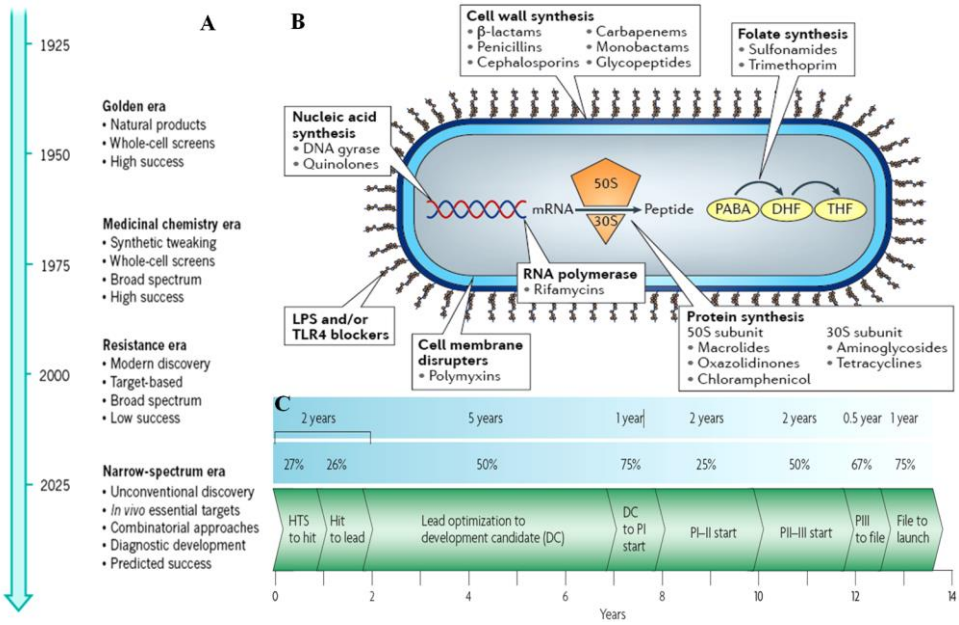


Figure 9: Resistance and drug-discovery. The models in antibiotic drug-discovery (A), important site of antibacterial actions [DHF, dihydrofolic acid; LPS, lipopolysaccharide; PABA, para-aminobenzoic acid; THF, tetrahydrofolic acid; TLR4, Toll-like receptor 4] (B), and timelines in development of broad-spectrum antibacterial and estimated success metrics.

Revolutionizing Antibiotic Discovery Against S. aureus: Overcoming Challenges and Exploring Novel Approaches

The introduction of antibiotics in the 1940s and 1950s revolutionized the treatment of bacteraemia caused by *S. aureus* and Streptococcus pneumonia, which previously had low survival rates. The development of new antibiotics against these pathogens entered the market as antimicrobial agents in the 1950s and 1960s. Unfortunately, these developed leads treated against eradication, and the high cost of research and difficulties in entering the discovery of antimicrobial drugs with unexplored modes of action have continuously discouraged pharmaceutical companies from the area of drug discovery. However, the market size of discovering antimicrobials has increased to about US $25 billion per year due to the emergence of resistant microbial strains that are less effective against already existing drugs in nature. Various classes of antimicrobial agents have been developed to treat *S. aureus* infections, including cephalosporins, quinolones, aminoglycosides, clindamycin, chloramphenicol, tetracyclines, oxazolidiones, trimethoprim, and sulphonamides (Table 7). These classes of antimicrobials work differently on bacterial metabolism, inhibiting cell wall synthesis, DNA DNA gyrase, protein synthesis, enzymes, and other targets. In the past decade, Roche, GlaxoSmithKline, Bristol-Myers Squibb, and Eli Lilly were leading pharma and biotech companies that started research on antimicrobial agents. However, nowadays, most of these companies have left the business due to the emergence of resistant strains to newly introduced drugs, which they viewed as a hurdle and unprofitable. This has discouraged the development of new antimicrobials, which could end in few years without profit. Despite this, several drugs are still earning more than US $1 billion per year, such as Augmentin (amoxicillin/clavulanate potassium; Glaxo- SmithKline), Cipro (ciprofloxacin hydrochloride; Bayer), and Zithromax

(azithromycin; Pfizer). The emergence of antibiotic resistance in S. aureus and other bacterial pathogens is a significant concern, and the lack of new antibiotic discoveries is a major challenge. To combat this issue, new approaches and strategies for antibiotic discovery are required, such as identifying unconventional targets and using narrow-spectrum agents. Efforts to develop new antibiotics require a concerted effort between medicinal chemistry and biological sciences, and investment in such programs remains critical to combat antibiotic resistance and emerging infectious diseases [117-120].

Table 7: The main classes of antibiotics and their examples.

Class	**Examples**
β-Lactams	
Penicillins	Penicillin G, penicillin V, methicillin, oxacillin, cloxacillin,
	dicloxacillin, nafcillin, ampicillin, amoxicillin, carbenicillin,
	ticarcillin, mezlocillin, piperacillin, azlocillin, temocillin
Cephalosporins	
First generation	Cepalothin, cephapirin, cephradine, cephaloridine, cefazolin
Second generation	Cefamandole, cefuroxime, cephalexin, cefprozil, cefaclor,
	loracarbef, cefoxitin, cefmetazole
Third generation	Cefotaxime, ceftizoxime, ceftriaxone, cefoperazone,
	ceftazidime, cefixime, cefpodoxime, ceftibuten, cefdinir
Fourth generation	Cefpirome, cefepime
Carbapenems	Imipenem, meropenem
Monobactams	Astreonam
β-Lactamase inhibitors	Clavulanate, sulbactam, tazobactam
Aminoglycosides	Streptomycin, neomycin, kanamycin, paromycin, gentamicin,
	tobramycin, amikacin, netilmicin, spectinomycin, sisomicin,
	dibekalin, isepamicin
Tetracyclines	Tetracycline, chlortetracycline, demeclocycline, minocycline,
	oxytetracycline, methacycline, doxycycline
Rifamycins	Rifampicin (also called rifampin), rifapentine, rifabutin,
	bezoxazinorifamycin, rifaximin
Macrolides	Erythromycin, azithromycin, clarithromycin
Lincosamides	Lincomycin, clindamycin
Glycopeptides	Vancomycin, teicoplanin
Streptogramins	Quinupristin, daflopristin
Sulphonamides	Sulphanilamide, *para*-aminobenzoic acid, sulfadiazine,
	sulfisoxazole, sulfamethoxazole, sulfathalidine
Oxazolidinones	Linezolid
Quinolones	Nalidixic acid, oxolinic acid, norfloxacin, pefloxacin,
	enoxacin, ofloxacin/levofloxacin, ciprofloxacin,
	temafloxacin, lomefloxacin, fleroxacin, grepafloxacin,
	sparfloxacin, trovafloxacin, clinafloxacin, gatifloxacin,
	moxifloxacin, sitafloxacin
Others	Metronidazole, polymyxin, trimethoprim

Repurposing FDA-Approved Non-Antimicrobial Drugs as Antimicrobials: A Promising Approach for Discovering New Antibiotics Against MRSA

The emergence of microbial resistance to antibiotics has become a significant global threat, and there is a need for the discovery of new antimicrobials to address this public health crisis. The development of resistance to antibiotics has led to the need for costly and toxic alternatives to treat infections caused by resistant pathogens. In addition, the emergence of resistant ESCAPE pathogens and the development of new resistance mechanisms have made the situation more critical. The Food and Drug Administration (FDA) has searched 727 approved non-antimicrobial drugs for their antimicrobial activity against ESCAPE pathogens, leading to the discovery of new drug candidates. However, additional drugs must be screened further to elucidate their potential clinical applications, efficacy, safety, toxicity, and pharmacokinetic parameters before they are approved for commercialization. This leads to an understanding of the appropriate route of administration to bypass low cost, the time associated, and resistance to the identified target to place this drug in the market place. To accelerate the development of new antibiotics, about 1,600 FDA-approved drugs were screened, including some clinically safe molecules that have not yet been proven to be used as antimicrobial agents. Among them, 48 small molecules were presented with minimum inhibitory concentrations (MIC) against MRSA (Table 7). The use of non-antimicrobial agents as antimicrobial agents represents an untapped source of new antibiotic candidates that can save expensive research. These small molecular weight molecules suggest a new avenue for drug discovery programs. Once the molecular targetfor the respective drug is identified, it can be sub-structured to enhance its potential antibiotic properties without altering its drug-like properties. The repurposing of non-antimicrobial drugs as antimicrobial agents is a promising approach for discovering new antibiotics to combat antibiotic resistance. The FDA's search for new drug candidates has highlighted the potential of repurposing existing drugs. However, identifying the molecular target of these drugs and optimizing their properties for clinical use poses significant challenges. Efforts to develop new antibiotics must continue to address the public health crisis caused by antibiotic-resistant pathogens. The discovery of new antimicrobial agents is critical to combat antibiotic resistance, and the repurposing of non-antimicrobial drugs as antimicrobial agents is a promising approach [121-124].

Teixobactin: A Promising New Antibiotic Against *S. aureus*

The discovery of new antibiotics is an expensive business, and the pharmaceutical industry's enthusiasm for discovering new drug candidates has decreased. The challenge remains to select a starting compound for the discovery phase of antibiotic development. Currently available antibiotics were mostly discovered during the golden age of antibiotics (1940-1960s), and efforts to discover new drugs are becoming less productive. However, one study discovered a promising compound against *S. aureus* from a Gram-negative β-proteobacterium named Elephtheria terrae (Fig. 10A). The compound, named teixobactin, was purified and its structure elucidated. Teixobactin represents a new chemical scaffold that is different from existing antibiotics. Teixobactin acts by binding to bacterial cell-wall polymers precursors, including lipid II (peptidoglycan) and lipid III (teichoic acid). This confirms its specificity and efficacy against Gram-positive bacteria, which have a thick peptidoglycan containing teichoic acid, but less sensitivity against Gram-negative bacteria due to the presence of an impermeable outer membrane that prevents access to lipid II, which lacks teichoic acid. The lack of resistance to teixobactin is attributed to its targeting of lipid molecules involved in cell-wall synthesis rather than proteins. Teixobactin's efficacy against *S. aureus* and lack of resistance make it an attractive candidate for further development as a new antibiotic. The discovery of teixobactin also highlights thepotential of untapped soil microorganisms as a source of new antibiotic scaffolds. The study's findings suggest that there are still many soil microorganisms that remain undiscovered, and efforts to mine them for new antibiotics could lead to the discovery of novel compounds. Teixobactin's efficacy against *S. aureus* and lack of resistance is comparable to that of vancomycin, which also targets lipid molecules involved in cell-wall synthesis and has not shown any resistance strains since its discovery in 1953. The discovery of teixobactin represents a new avenue for drug discovery programs and could lead to the development of new antibiotics to combat antibiotic resistance [124-126].

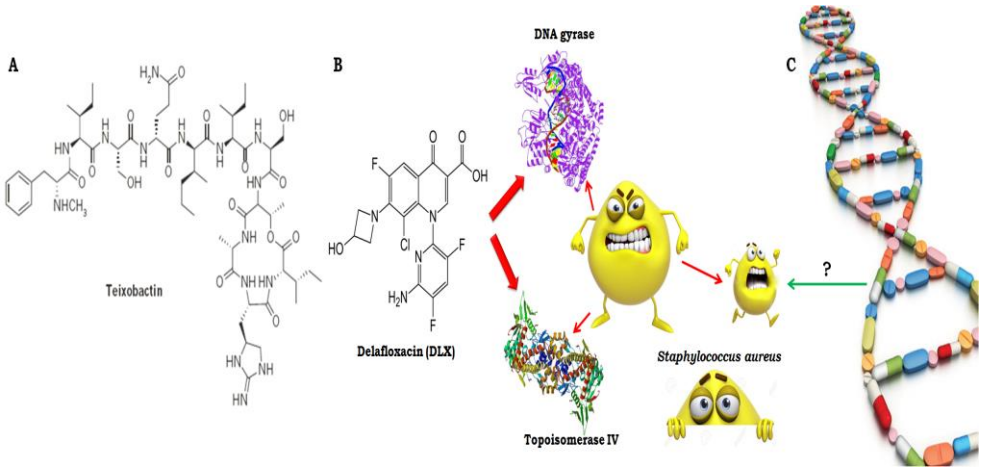


Figure 10: Past, present and future in antibiotics drug-discovery. The structure of antibiotic teixobatin isolated from bacterium *Elephtheria terrae* against *S. aureus* **(A)**, new antibiotic delafloxaci (DLX) with novel mechanism against *S. aureus* acute bacterial skin and skin structure infection (ABSSSI) by acting on DNA gyrace and topoisomerase IV **(B)**, and hope on biological for future *S. aureus* treatment **(C)**.

Delafloxacin: A New Weapon Against MRSA and Bacterial Infections

Delafloxacin (DLX) is the one such recently approved by Food Drug Administration (FDA) with broad antimicrobial, attracted for therapeutic option due favourable pharmacokinetic and pharmacodynamic profile (Fig. 10B) [127]. The DLX is a fluoroquinolone (FQ) antibiotic developed and currently, marketing by Melinta Therapeutics and Cempra in the trade name of BAXDELA shaving excellent spectrum against a wide range of clinically important Gram-positive (*Staphylococcus aureus, S. haemolyticus, S. lugdunensis, S. pyogenes, S. agalactiae, Streptococcus anginosus Group, and Enterococcus faecalis, and* Gram-negative *Escherichia coli, Enterobacter cloacae, Klebsiella pneumonia, and Pseudomonas aeruginosa* pathogens. Among the pathogens DLX active against methicillin-resistant and methicillin-sensitive *S. aureus* (MRSA and MSSA) [128]. Delafloxacin (DLX) is a recently approved broad-spectrum antimicrobial drug that has shown promising therapeutic potential due to its favorable pharmacokinetic and pharmacodynamic profile (Table 6). In June 2017, the US Food and Drug Administration (FDA) approved DLX for use in treating bacterial infections (Fig. 10B). DLX is a fluoroquinolone (FQ) antibiotic that was developed by Melinta Therapeutics and Cempra and is marketed under the trade name BAXDELA. It has an excellent spectrum of activity against a wide range of clinically important Gram-positive bacteria, including *Staphylococcus aureus*, *S. haemolyticus, S. lugdunensis, S. pyogenes, S. agalactiae, Streptococcus anginosus* Group, *and Enterococcus faecalis*, as well as Gram-negative bacteria such as *Escherichia coli, Enterobacter cloacae, Klebsiella pneumonia,* and *Pseudomonas aeruginosa*. DLX has been shown to be active against both methicillin-resistant and methicillin-sensitive *S. aureus* (MRSA and MSSA), which are major causes of healthcare-associated infections and community-acquired infections. DLX's broad-spectrum activity and effectiveness against MRSA make it an attractive candidate for the treatment of a range of bacterial infections. DLX's mechanism of action involves inhibiting the bacterial DNA gyrase and topoisomerase IV enzymes, which are essential for bacterial DNA replication and cell division. This results in the inhibition of bacterial growth and eventual bacterial cell death. DLX's favorable pharmacokinetic and pharmacodynamic properties allow for once-daily dosing and a short treatment duration. Additionally, DLX has shown good tissue penetration and distribution, including penetration into infected tissues such as skin and soft tissue. While DLX has shown promise as a new therapeutic option for bacterial infections, further studies are needed to evaluate its safety and efficacy in clinical practice. Nevertheless, DLX represents a promising addition to the arsenal of antibiotics available for treating bacterial infections.

The exceptional characteristics of DLX

DLX is a powerful fluoroquinolone antibiotic that has shown promise in treating life-threatening bacterial infections, including acute bacterial skin and skin structure infections (ABSSSIs), sepsis, and bacteremia. One of the reasons MRSA is resistant to some synthetic antibiotics is due to its tolerance for high pH. DLX, also known as 1-Deoxy-1(methylamino)-D-glucitol,1-(6-amino-3,5-difluoropyridin-2-yl)-8-chloro-6-fluoro-7-(3-hydroxyazetidin-1-yl)4-oxo-1,4dihydroquinoline-3-carboxylate (salt), has been found to have superior biocidal power compared to other fluoroquinolones. DLX's superior strength is due to three important structural differences in its carbon backbone. The chlorine moiety at C8 acts as an electron-withdrawing group, reducing the reactivity of the heterocycle and stabilizing the molecule. The C7 group acts as a weak acid, increasing its potency in acidic environments. Additionally, the presence of an aromatic ring attached to N1 greatly increases the molecular surface of DLX compared to other quinolone derivatives. DLX has broad-spectrum activity against a wide range of clinically important Gram-positive bacteria, including MRSA, as well as Gram-negative bacteria such as Escherichia coli, Enterobacter cloacae, Klebsiella pneumonia, and Pseudomonas aeruginosa. DLX's favorable pharmacokinetic and pharmacodynamic profile allows for once-daily dosing and a short treatment duration. DLX has also shown good tissue penetration and distribution, including penetration into infected tissues such as skin and soft tissue. The mechanism of action of DLX involves inhibiting bacterial DNA gyrase and topoisomerase IV enzymes, which are essential for bacterial DNA replication and cell division. This results in the inhibition of bacterial growth and eventual cell death. Several studies have demonstrated the efficacy of DLX in treating bacterial infections, including those caused by MRSA. In a randomized, double-blind, phase 3 trial, DLX was found to be non-inferior to vancomycin and aztreonam in treating ABSSSIs. DLX also demonstrated superiority in treating infections caused by MRSA compared to vancomycin and linezolid. While DLX has shown promise as a new antibiotic for treating bacterial infections, further studies are needed to evaluate its safety and efficacy in clinical practice. Nevertheless, DLX represents a promising addition to the arsenal of antibiotics available for combating bacterial infections, including those caused by MRSA [129-131].

Unleashing the Power of Analog Positioning: The Story of DLX and Its Potent Antibacterial Efficacy

The importance of the analog position in delafloxacin (DLX) has been shown to play a crucial role in its efficacy as an antibacterial agent. Other fluoroquinolones such as levofloxacin (LVX), ciprofloxacin (CPX), and moxifloxacin (MXL) have demonstrated that the presence of important chemical moieties and their role in the biological system can drive the overall compound's efficacy by changing its character according to the surrounding environment. DLX lacks the C7 basic group, which makes it a zwitterion with two ionized groups and no net charge on the molecule. This property gives DLX a weak acid character (pKa 5.4), which is lower than other quinolones, allowing it to penetrate the phagolysosome (pH 5-5.5) (Fig. 10B). The neutral or ionized form of DLX enables it to cross the transmembrane into the bacterium in a gradient fashion [131-134]. Inside the bacteria, where the pH is neutral, DLX is deprotonated to retain in an ionic form, resulting in higher transmembrane passage and retention within the bacteria, compared to moxifloxacin (MXF), which is retained in a zwitterionic form to a lesser extent and returns to the exterior of the bacteria [132]. This property of DLX makes it highly effective in an acidic medium compared to other quinolones. Studies have shown that DLX is highly effective against *S. aureus*, even at acidic conditions such as empyema and abscesses, urine, the stomach, and the vagina, where the pH is usually low, indicating its in vivo compatibility at infection sites [132]. However, it is essential to note that the acidic pH may also affect other vital functions of transport mechanisms in the body system. The efficacy of DLX has been compared at pH 5.5 and pH 7.4 against *S. aureus*, and the results showed that the DLX efficacy was 5-7 dilutions lesser at pH 5.5 than at pH 7.4, indicating higher values of 0.00003 µg/mL [132,134-136]. This supreme activity of DLX justifies its potential use in treating infections at acidic conditions. DLX's unique chemical structure and mechanism of action distinguish it from other potent antibacterial compounds such as β-lactams, macrolides, aminoglycosides, glycopeptides, oxazolidinones, and tetracyclines. DLX is a fourth-

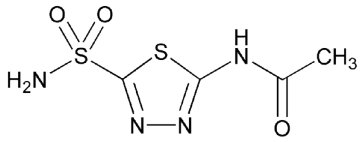
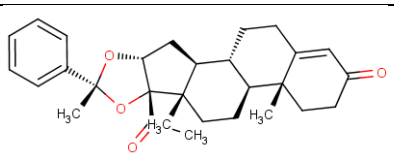
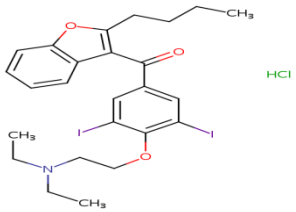
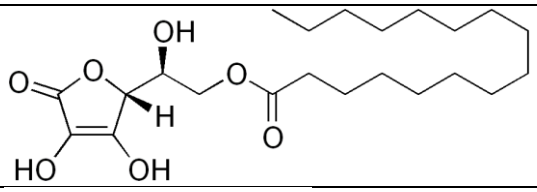
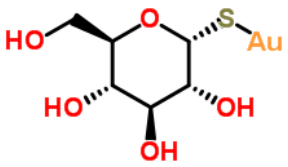
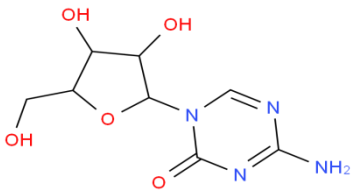
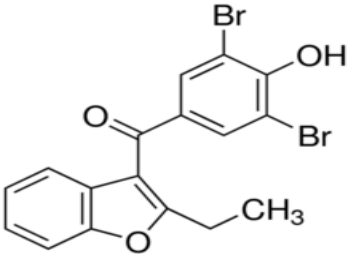
generation fluoroquinolone (FQ) due to its dual action at DNA gyrase (topoisomerase II) and topoisomerase IV, which slows down the development of resistance in *S. aureus* by interfering with cell DNA replication, transcription, repair, and recombination. Resistance to FQ, including DLX, occurs only if there is a mutation at specific regions of the target enzymes DNA gyrase and topoisomerase IV, referred to as quinolone-resistance determining regions (QRDRs) or through altered efflux. Multiple mutations at QRDRs have been attempted to develop resistance to DLX in both Gram-positive and Gram-negative bacteria, but in vitro studies have shown that DLX-resistant strains are selected at a frequency of <10^-9. In vitro DLX investigations in the United States and Europe in 2014 reported DLX comparative to available previous drugs against MRSA and MSSA, indicating the potent and promising nature of DLX against *S. aureus* infections and diseases (Table 8) [133-139]. In conclusion, the analog position in DLX plays a crucial role in its efficacy as an antibacterial agent, and it targets bacterial type II topoisomerase, DNA gyrase, and topoisomerase IV, making it distinct from the currently used antibiotics. The DLX dual-targeting mechanism avoids twin objectives, such as cross-resistance and emergence of de novo resistance, making it a potent and promising drug against *S. aureus* infections and diseases [140]. However, more research is needed to understand the potential side effects and long-term effects of DLX in the human body.

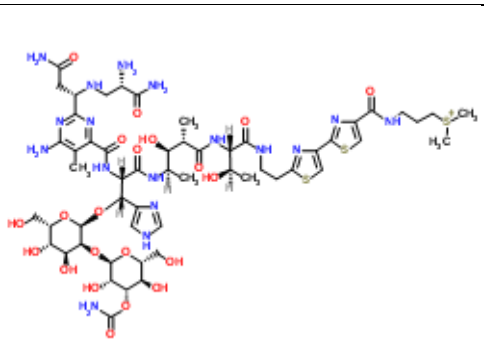
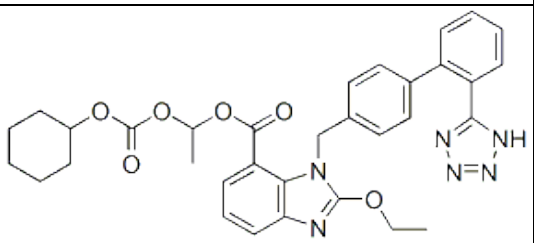
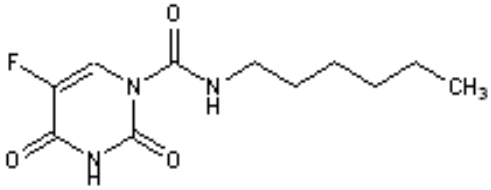
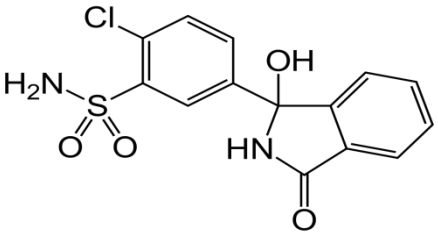
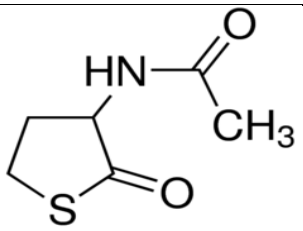
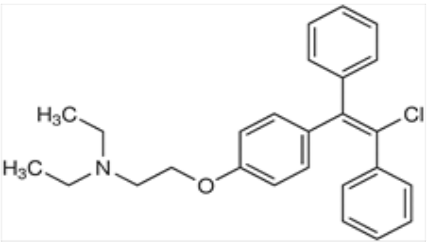
DLX: An Anionic Fourth-Generation Fluoroquinolone with Dual-Action Mechanism to Combat Antibiotic-Resistant *S. aureus* Infections

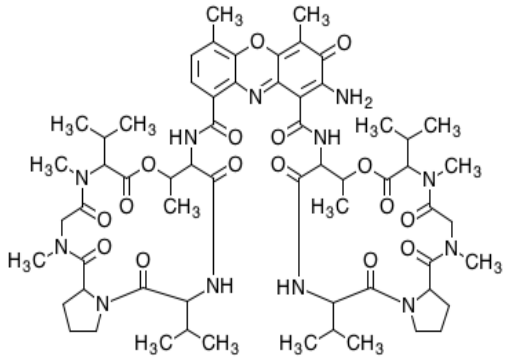
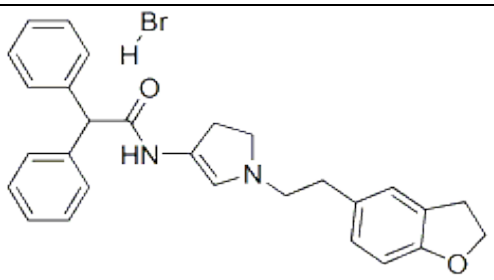
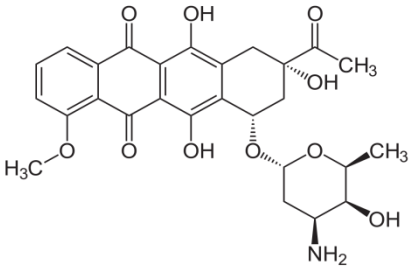
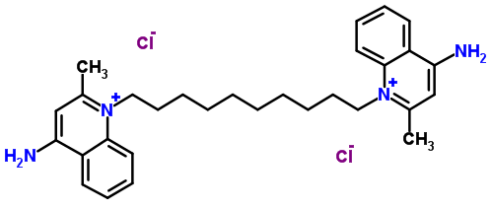
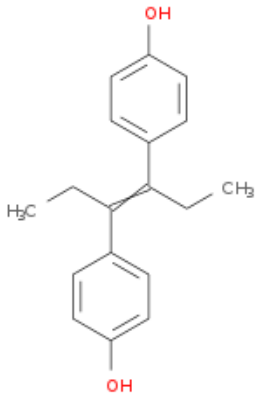
S. aureus has developed several mechanisms to survive against potent antibiotics, including resistance through horizontal gene transfer, biofilm formation, and secretion of antibiotic modulator β-lactamase, which alters the antibiotics' structure by hydrolyzing the common molecular structure in all antibiotics, making them incapable against *S. aureus*. The main bacterial mechanism of antibiotic resistance includes activation of the efflux pump, modifications of the drug target, enzymatic inactivation of drugs, and decreased uptake of antibiotics by changes in outer membrane permeability [141]. There is an urgent need for new antibacterial agents to counter multi-drug resistant infections. Interestingly, bacterial type II topoisomerase, DNA gyrase, and topoisomerase IV are suitable targets for the development of antibiotic drugs, as evidenced by the fluoroquinolone (FQ) and aminocoumarin classes [142,143]. DLX is a fourth-generation FQ and has a dual-targeting mechanism distinct from the existing antibiotics to avoid twin objectives such as cross-resistance and emergence of de novo resistance. DLX is a potent antibacterial drug with an anionic nature. Detailed studies have revealed that DLX is a dose-dependent, dual-action inhibitor of DNA gyrase (topoisomerase II) and topoisomerase IV, which slows down the development of resistance in *S. aureus* by interfering with cell DNA replication, transcription, repair, and recombination [144]. Resistance to FQ, including DLX, occurs only if there is a mutation at specific regions of the target enzymes DNA gyrase and topoisomerase IV, referred to as quinolone-resistance determining regions (QRDRs), or through altered efflux. DLX has a different chemical structural anatomy and mechanism compared to other potent antibacterial compounds such as β-lactams, macrolides, aminoglycosides, glycopeptides, oxazolidinones, and tetracyclines. Attempts have been made to develop resistance to DLX by multiple mutations at QRDRs in both Gram-positive and Gram-negative bacteria. However, in vitro studies have shown that DLX-resistant strains are selected at a frequency of <10^-9. In vitro DLX investigations conducted in the United States and Europe in 2014 reported DLX's comparability to previously available drugs against methicillin-resistant *S. aureus* (MRSA) and methicillin-sensitive *S. aureus* (MSSA), indicating its potent and promising nature against *S. aureus* infections and diseases (**Table** 8) [145]. In conclusion, *S. aureus* has developed several mechanisms to resist antibiotics, making the development of new antibacterial agents like DLX necessary. DLX's dual-targeting mechanism of DNA gyrase and topoisomerase IV makes it a potent and promising drug against *S. aureus* infections and diseases. The attempts to develop resistanceto DLX through QRDR mutations have been unsuccessful, indicating its potential effectiveness in treating drug-resistant infections. Further research is needed to explore the potential side effects and long-term effects of DLX on human health. DLX's unique chemical structure and mechanism of action distinguish it from other potent antibacterial compounds, making it a valuable addition to the current arsenal of

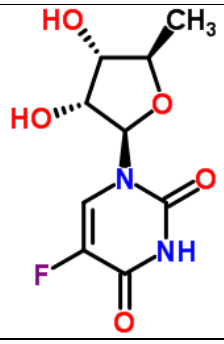
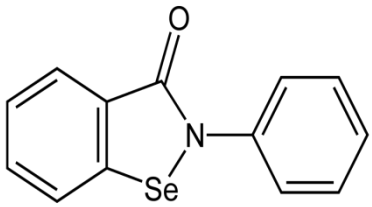
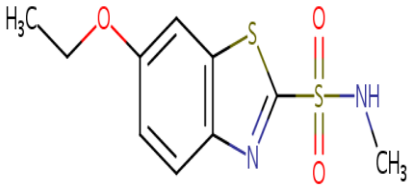
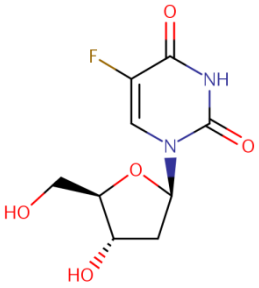
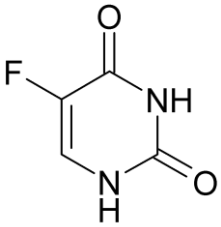
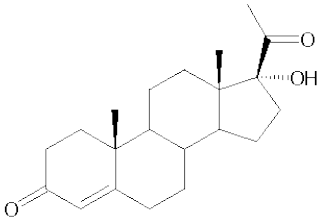
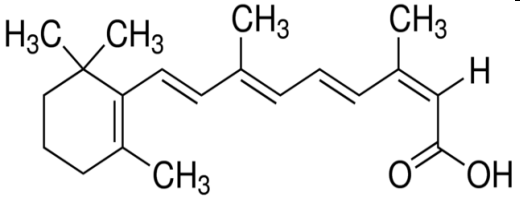
antibiotics. DLX's efficacy against *S. aureus* infections and diseases is promising, but more clinical studies are needed to establish its safety and efficacy in treating a range of bacterial infections.

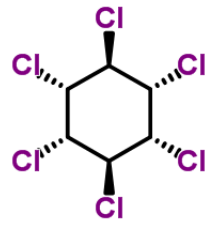
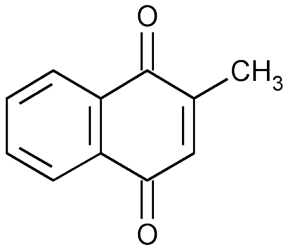
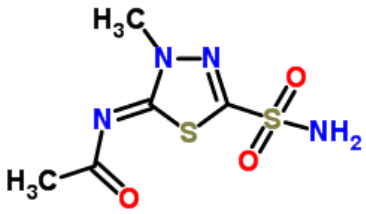
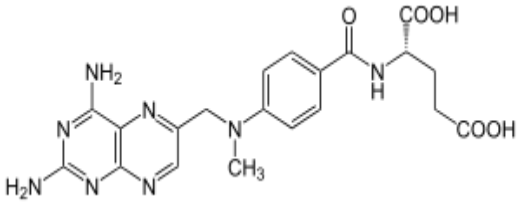
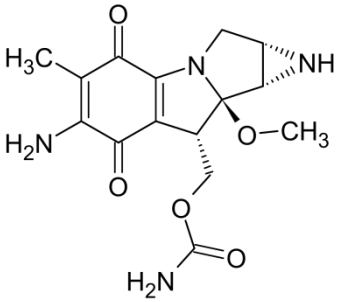
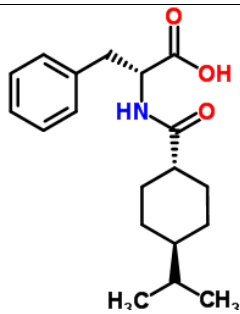
Table 8: The description and MIC of non-antimicrobial compounds against Gram-positive MRSA.

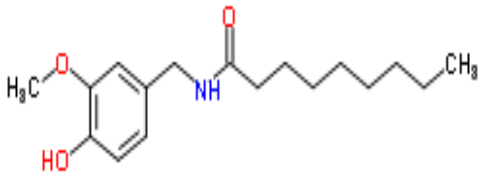
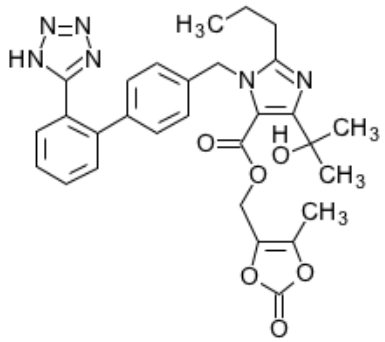
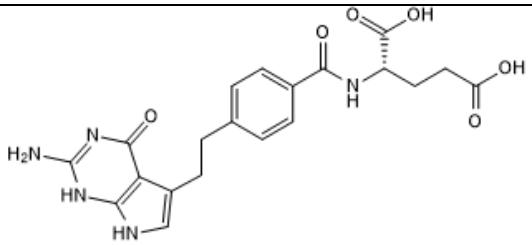
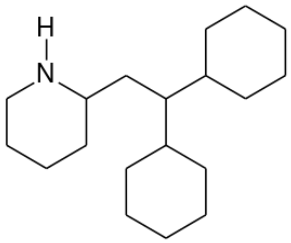
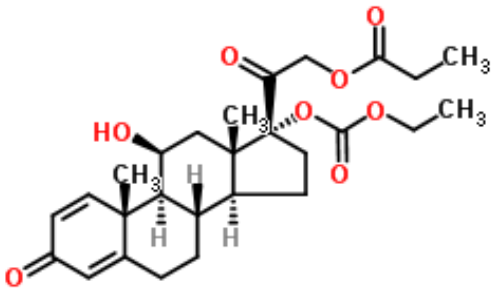
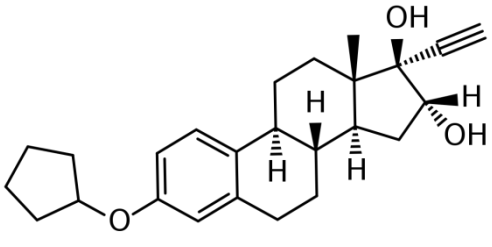
Sl. No.	Compound name	Description	Structure	MIC (μM)
1	Acetazolamide	carbonic anhydrase inhibitor, diuretic, antiglaucoma		>16
2	Algestone acetophenide	antiacne, rogestin		>16
3	Amiodarone hydrochloride	adrenergic gonist, coronary vasodilator, Ca channel blocker		>16
4	Ascorbyl palmitate	antioxidant		>16
5	Aurothioglucose	antirheumatic		>16
6	Azacitidine	antineoplastic,pyrimidine antimetabolite		>16
7	Benzbromarone	uricosuric		16

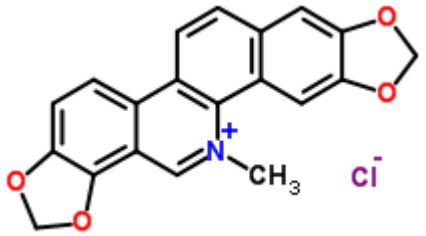
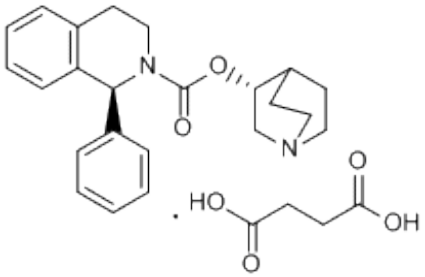
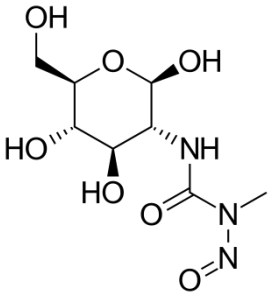
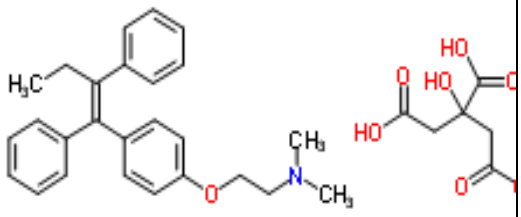
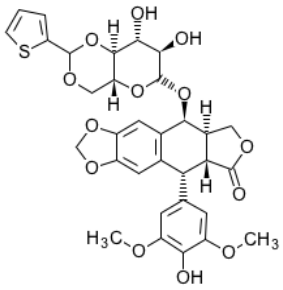
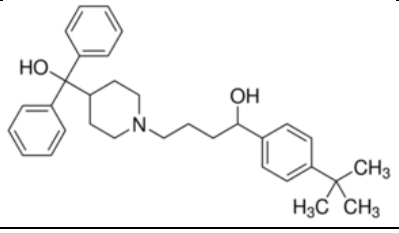
8	Bleomycin	antineoplastic		4
9	Candesartan cilexetil	angiotensin 1 receptor antagonist		16
10	Carmofur	antineoplastic		4
11	Chlorthalidone	diuretic, antihypertensive		>16
12	Citiolone	hepatoprotectant, free radical scavenger		>16
13	Clomiphene	anti-estrogen		>16

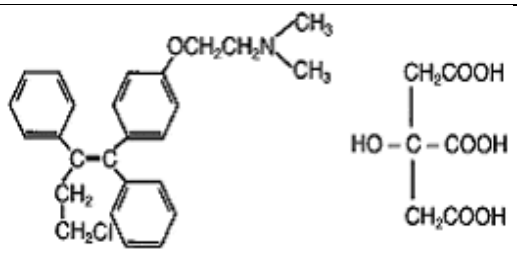
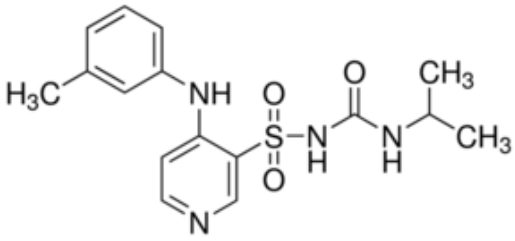
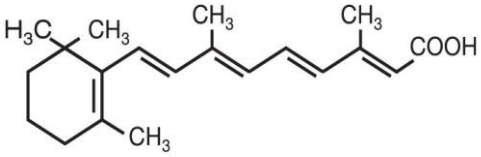
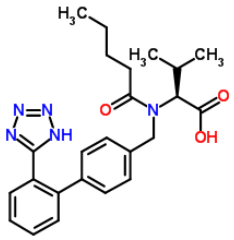
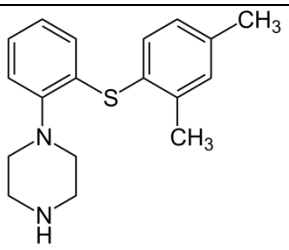
14	Dactinomycin	antineoplastic, intercalating agent		0.5
15	Darifenacin hydrobromide	M3 muscarinic antagonist, bladder suppressant		>16
16	Daunorubicin	antineoplastic		16
17	Dequalinium chloride	antiseptic wound dressings and mouth infections		>16
18	Diethylstilbestrol	estrogen		16

19	Doxifluridine	antineoplastic, pyrimidine antimetabolite		2
20	Ebselen	antioxidant, lipoxygenase inhibitor, inhibits oxidation of LDL		0.5
21	Ethoxzolamide	carbonic anhydrase inhibitor, antiulcer, antiglaucoma		>16
22	Floxuridine	antineoplastic, antimetabolite		<0.0078
23	Fluorouracil	antineoplastic, pyrimidine antimetabolite		0.5
24	Hydroxyprogesterone	progestin		>16
25	Isotretinon	antiacne, antineoplastic		>16

26	Lindane	topical treatment of lice and scabies		16
27	Menadione	prothrombogenic agent		16
28	Methazolamide	carbonic anhydrase inhibitor		>16
29	Methotrexate	antineoplastic, antirheumatic, folic acid antagonist		>16
30	Mitomycin	antineoplastic		16
31	Nateglinide	antidiabetic		>16

32	Nonivamide	analgesic (topical), depletes Substance P		>16
33	Olmesartan medoxomil	angiotensin II inhibitor prodrug, antihypertensive		>16
34	Pemetrexed	antineoplastic, thymidylate synthase inhibitor		>16
35	Perhexiline	calcium channel blocker, coronary vasodilator		>16
36	Prednicarbate	antiinflammatory, glucocorticoid		8
37	Quinestrol	estrogen		>16

38	Sanguinarium chloride	antineoplastic, antiplaque agent		4
39	Solifenacin succinate	muscarinic M3 antagonist		16
40	Streptozotocin	antineoplastic, alkylating agent		8
41	Tamoxifen citrate	antineoplastic, antiestrogen		16
42	Teniposide	antineoplastic		4
43	Terfenadine	antihistaminic		16

44	Toremifene citrate	estrogen antagonist, antineoplastic		>16
45	Torsemide	diuretic, inhibits Na/K/2Cl carrier system		>16
46	Tretinoin	keratolytic, antiacne, antineoplastic		>16
47	Valsartan	angiotensin II inhibitor, antihypertensive		>16
48	Vortioxetine	antidepressant		>16

DLX - A Dual-Action Fluoroquinolone for ABSSSI Caused by MRSA: Efficacy and Safety Considerations

DLX, a potent antibacterial agent, is recommended for the treatment of acute bacterial skin and skin structure infections (ABSSSI) caused by bacterial pathogens, including methicillin-resistant Staphylococcus aureus (MRSA), through both oral and intravenous (IV) administrations. The New Drug Applications (NDA) for delafloxacin (Baxdela) 450 mg tablets and 300 mg injections were approved by the FDA. However, a five-year surveillance study needs to be conducted to document resistance (within 2022), and IV distribution needs to be determined in pregnant rats due in 2018. DLX has a favorable pharmacodynamic profile, including cardiac electrophysiology and photosensitivity potential, and pharmacokinetics, including absorption, distribution, elimination, metabolism, excretion, drug interactions, and transporters, making it an attractive therapeutic option against MRSA infections in the context of ABSSSI. Despite the good safety profile of DLX, long-term use needs to be followed up due to serious effects observed in other compounds within this class that were withdrawn [146]. DLX covered two phase 3 trials (referred to as PROCEED) by a comparator with vancomycin + aztreonam, following the first trial IV-only (NCT01811732) and the second ABSSSI trial IV/oral (NCT01984684). The efficacy of DLX passed both trials by demonstrating non-interference with the comparator, but diarrhea and nausea were the most frequent adverse effects documented during treatment [147]. DLX has initiated phase 3 programs in hospital-treated patients (NCT02679573) and further plans for the treatment of complicated urinary tract infections (cUTI) [147]. DLX's successful completion of phase 3 trials and its FDA approval for the treatment of ABSSSI caused by MRSA represent a significant milestone in the development of novel antibacterial agents . However, close monitoring of long-term use is necessary due to the withdrawal of other compounds in this class, indicating the potential for serious side effects. The documented adverse effects of DLX, such as diarrhea and nausea, need to be addressed to enhance patients' adherence to the therapy [148]. Further clinical studies are needed to evaluate the safety and efficacy of DLX against a wide range of bacterial infections, including cUTI. In conclusion, DLX represents a promising therapeutic option for the treatment of ABSSSI caused by MRSA. Its dual-targeting mechanism of DNA gyrase and topoisomerase IV, along with its unique chemical structure, distinguishes it from other potent antibacterial compounds. The successful completion of phase 3 trials and FDA approval of DLX demonstrate its efficacy and safety profile (Table 9). However, long-term use needs to be monitored, and adverse effects need to be addressed to enhance patients' adherence to the therapy [146-153]. Further clinical studies are needed to establish the safety and efficacy of DLX in treating a wide range of bacterial infections.

Table 9: Gram-positive and Gram-negative active of delafloxacin and comparator agents.

Antibacterial Agent	Gram-positive Activity	Gram-negative Activity
Delafloxacin	++++	+++
Levofloxacin	++	++
Moxifloxacin	+++	++
Ciprofloxacin	+	++
Vancomycin	++++	-
Linezolid	++++	-
Daptomycin	++++	-

The Gram-positive and Gram-negative activity of different antibacterial agents, including delafloxacin and several comparator agents. The Gram-positive and Gram-negative activity of each agent is rated

on a scale of 0 to 4, with 0 indicating no activity and 4 indicating very high activity. ++++: Very high activity, +++: High activity, ++: Moderate activity, +: Low activity, - : No activity>

Challenges and Opportunities in the Development of Effective Diagnostic Techniques and Novel Antibiotics to Overcome Antimicrobial Resistance in *S. aureus*

Many molecular methods are available to detect the target in a sample of interest and prescribe treatment to avoid infection further. In this regard, an important advancement in our understanding of S. aureus resistance and many challenges are still remains to solve. Still many molecular methods and higher MALDI-Biotyper version in diagnosis, do not have particularly to monitor resistance level, detect particular mutations, detect changes in efflux pump, membrane permeability, and virulence factors [154]. Therefore, there is a need for effective diagnostic technique for identifying the target in an infected sample to treat in future. Molecular machines (MM) in both human and pathogen has been crafted by evolution, but how drug developer can disable the multicomponent programs of MMs [155]. By 2050, the predicted death due to antimicrobial resistance (AMR) is higher than cancer, and it demands remarkable novel modes of new antibiotics to combat wilful by spreading classic therapeutic candidates (http://amr-review.org/). Importantly in all sets of drug discovery programs (DDP), no matter how biologist logically underpinning and what are the stringent scientific efforts needed to select a suitable target [156]. But, a need of clear information of 'in' techniques (in vitro, in vivo, and in silico) to deal indeterminable diseases associated with it [157]. Later in drug discovery (DD) events, play a blend of unknown serendipity and continuous ingenuity on the edge between science and art [158]. For only the sake of clarity, the DDP does not consider in a retrospective scientific report, particularly those mention medicinal chemistry programs. Sometimes, the existing reports fail the dogmas; can be really indicating for the true story of DD. This is our humble opinion; in the case of few accompany papers in the literature [159]. Some of the key points are highlighted to avoid and overcome the resistance in microbes such as 1. Understanding commensal biology and pathogen interaction with the host [160], 2. Increased epidemiology and surveillance of resistance [161], 3. Use of alternative novel antimicrobials and their prudent utilization[162], 4. Safe use of antimicrobials through cycling, combination and restriction [163], 5. Improved hygiene[164], 6. Banning of nontherapeutic candidates [165], 7. Improve the education of public and care specialist[166], 8. Decrease the bactericide use [167], and 9. Must use alternative strategies to avoid the emergence of resistant strains to avoid future public burden [168]. The DNA gyrase and topoisomerase enzymes are involved in the introducing negative supercoils and decatenation of DNA [169]. The high conservative similarity between DNA gyrase and topoisomerase IV promotes and offers a promising multitargeting single pharmacophore in the prospect of future [170]. The numbers of quinolone-based drugs are targeted for topoisomerase II including eukaryotic due to quinolones C7 position containing an aromatic substituent [171]. Still the resistance to quinolones classes, the is an alternative option of type II topoisomerases to provide a versatile platform for future antibacterial discovery for novel chemical ligands in DD to explore the novel binding interaction with target enzymes to bypass the mutations associated resistance in bacteria [172]. To overcome this problem, fourth generation FQ are emerged to selective binding to topoisomerase IV and to enhanced for Gram-positive coverage [173]. A new upcoming trend can minimize the emergence and impact of *S. aureus* resistance to antibiotics through use of 'antibiotic adjuvant' (can also call as adjuvant potentials or adjuvant breakers) therapy combined with an antibiotic [174]. They have little or zero antibiotic function only co-administered with other antibiotic having block core bacterial mechanism of resistance or efficient antibacterial activity [175]. This booming and successful strategy is the best-focused area for future drug discovery field to face the challenge of multi-drug resistance [176]. Among currently used promising strategies to unlock the spread of multi-drug resistance worldwide, the use of 'antibiotic adjuvant' along with antibiotic has proven its efficacy in clinical trials [177]. This approach can overcome lifespan of drug and disable the challenge for developing novel chemical entities of unexpected bacterial targets can be relaxed through challenge activities of drug discovery programs [178]. Finally, the ability to predict resistance mechanisms before clinical testing would prove essential in the successful, controlled use of this powerful antibiotic [179]. In this regard, exploring the strategies

for drug-resistance in clinically relevant bacterial strains (MRSA) would provide vital clues into potential resistance liabilities in the future [180]. Through the careful analysis of DLX, focused chemical−biology approaches, and rigorous resistance studies, there is hope that DLX can be translated to lessons and rewards for future antibiotic drug therapy [181]. To enhance the antimicrobial potency of the drug and block the aforementioned prime mechanism to become antibiotic resistance, a great research view has been deviated towards explore the important bacterial pathogenicity involved in infection, and attenuated anti-virulence drugs to climb a step of clinical trials in near future [182-189].

Concluding remarks

Antibiotic resistance has become a significant limitation in our ability to effectively treat global health issues. The current projections indicate a concerning increase in the death rate from bacterial infections, with estimates suggesting a rise from 700,000 deaths per year to over 10 million by 2050 [186]. To address this critical issue, it is necessary to explore new mechanisms and novel drug candidates through interdisciplinary approaches. While many attempts to discover synthetic antibiotics have faced failures, natural sources have proven to be the most fruitful in antibiotic discovery. However, the brute force approach used in the past century has reached its limitations. The potential of the "not-yet-cultivated" microbial world has been tapped into through approaches that involve cultivating microorganisms and isolating highly potent compounds such as teixobactin, which irreversibly targets cell wall lipid precursors in *S. aureus* [190]. To maintain a pipeline of novel lead compounds, there is a need to explore alternative strategies. This necessitates applying a brute force approach through high-throughput genome sequencing and bioinformatic analyses of microbial genes and clusters, which can potentially unveil new antibiotics. The increasing speed and decreasing cost of DNA sequencing and synthesis hold promise for the future synthesis, cloning, and expression of millions of biosynthetic gene clusters in heterologous host assays for antibiotic activity. Biological approaches, including genomics and synthetic biology, will play a crucial role in the brute force discovery of novel compounds, serving the future needs of mankind [191]. This review not only highlights important foundational knowledge but also endeavors to address various host-pathogen interaction strategies. Understanding the protective mechanisms employed by *S. aureus* against the immune system is critical for the development of effective immune therapies or vaccines. Additionally, the review suggests overcoming resistance mechanisms through the discovery of new drug candidates in drug discovery programs. It also provides essential knowledge and clues for the development of new agonistic and antagonistic drugs to combat the treatment of notorious *S. aureus* infections, offering promising prospects for a healthier future [192]. In conclusion, the emergence and spread of antibiotic resistance have become significant limitations in our ability to effectively treat global health issues. To combat this issue, it is necessary to explore new mechanisms and novel drug candidates through interdisciplinary approaches. While the brute force approach used in the past century has reached its limitations, high-throughput genome sequencing and bioinformatic analyses of microbial genes and clusters hold promise for the future discovery of novel compounds. Biological approaches, including genomics and synthetic biology, will play a crucial role in serving the future needs of mankind [193].

Conclusion

In summary, research on *S. aureus* virulence mechanisms, host immunity responses, and antibiotic resistance has advanced greatly over the past two decades. However, resistant *S. aureus* strains continue to emerge and spread, representing an ongoing global health threat. While some recent successes and new drug candidates for resistant S. aureus have shown promise, continued research efforts are needed to develop effective treatments. Future directions for research include developing a more comprehensive understanding of *S. aureus* infection biology and pathogenesis, investigating host-pathogen interactions at the molecular level, identifying new antibiotic targets, and discovering and optimizing novel drugs with unique mechanisms of action. Combination therapies and host-directed therapies also show promise for combating resistant *S. aureus* strains. Improved diagnostics and vaccines could aid in treatment and prevention as well. With a dedicated, multi-pronged approach, the

scientific community stands a chance of gaining the upper hand against this challenging pathogen in the coming decades. However, coordinated efforts and increased funding for antibiotic research and development will be crucial to maximize opportunities for progress. Addressing the knowledge gaps and emerging research questions identified in this review through future studies will be instrumental in realizing the goal of effectively combating resistant *S. aureus* and thereby mitigating the threat it poses to global public health.

Declaration of interest

There are no conflicts of interests related to this manuscript.

References:

1. Ogston, A. (1881). On abscesses. The Lancet, 118(3036), 219-222.
2. Kluytmans, J., van Belkum, A., & Verbrugh, H. (1997). Nasal carriage of Staphylococcus aureus: epidemiology, underlying mechanisms, and associated risks. Clinical Microbiology Reviews, 10(3), 505-520.
3. Tong, S. Y., Davis,J. S., Eichenberger, E., Holland, T. L., & Fowler Jr, V. G. (2015). Staphylococcus aureus infections: epidemiology, pathophysiology, clinical manifestations, and management. Clinical Microbiology Reviews, 28(3), 603-661.
4. Becker, K., Roth, R., Peters, G., & Schumann, P. (2016). Reliable detection of Staphylococcus aureus from clinical samples by molecular methods. The Journal of Molecular Diagnostics, 18(6), 801-811.
5. Böcher, S., Skov, R. L., & Knudsen, J. D. (2016). A practical guide to diagnostic microbiology of Staphylococcus aureus. Frontiers in Microbiology, 7, 1-10.
6. Barber, M. (1961). Methicillin-resistant staphylococci. Journal of Clinical Pathology, 14(4), 385-393.
7. Jevons, M. P. (1961). "Celbenin"-resistant staphylococci. British Medical Journal, 1(5219), 124-125.
8. Pooja, S., & Gupta, S. (2020). Natural antimicrobials for controlling Staphylococcus aureus infections: A review. Journal of AppliedMicrobiology, 129(5), 1267-1283.
9. Otto, M. (2014). Staphylococcus aureus toxins. Current Opinion in Microbiology, 17, 32-37.
10. Tong, S. Y., Davis, J. S., Eichenberger, E., Holland, T. L., & Fowler Jr, V. G. (2015). Staphylococcus aureus infections: epidemiology, pathophysiology, clinical manifestations, and management. Clinical Microbiology Reviews, 28(3), 603-661.
11. Elizaquível, P., Aznar, R., & Sánchez, G. (2014). Recent developments in the use of viability dyes and quantitative PCR in the food microbiology field. Journal of Applied Microbiology, 117(1), 1-13.
12. Wang, X., Li, Y., Ouyang, P., & Lu, J. (2016). Identification of Staphylococcus aureus: traditional methods versus molecular methods. Molecular Medicine Reports, 14(4), 3429-3437.
13. Velusamy, V., Arshak, K., Korostynska, O., Oliwa, K., Adley, C., & Daniels, S. (2010). An overview of foodborne pathogen detection: in the perspective of biosensors. Biotechnology Advances, 28(2), 232-254.
14. van Belkum, A., Durand, G., Peyret, M., Chatellier, S., Zambardi, G., Schrenzel, J., ... & Dunbar, S. A. (2013). Rapid clinical bacteriology and its future impact. Annals of Laboratory Medicine, 33(1), 14-27.
15. Patel R. MALDI-TOF MS for the diagnosis of infectious diseases. Clin Chem 2015;61(1):100-11.
16. Bhatt, V., et al. (2018). A review on matrix assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF MS): A versatile tool for microorganism identification and diagnosis. Journal of Microbiological Methods, 151, 27-41.
17. Forbes, B. A., et al. (2017). Practice guidelines for clinical microbiology laboratories: matrix-assisted laser desorption ionization time-of-flight mass spectrometry. Clinical Microbiology Reviews, 30(1), 1-29.

18. Jorgensen, J. H., et al. (2015). Techniques for identification of microorganisms. Manual of Clinical Microbiology, 10, 191-212.
19. Jamal, W., et al. (2013). Comparative evaluation of the VITEK 2, MicroScan, and Phoenix systems for rapid identification and susceptibility testing of clinical isolates of Staphylococcus aureus. Diagnostic Microbiology and Infectious Disease, 76(3), 392-397.
20. Martiny, D., et al. (2012). Evaluation of the MALDI Biotyper for identification and antimicrobial susceptibility testing of Gram-positive rods—a development towards one-day diagnostics? PLoS One, 7(12), e38490.
21. Alatoom, A. A., et al. (2011). Comparison of direct colony method versus extraction method for identificationand antimicrobial susceptibility testing of bacteria using the MALDI Biotyper. Journal of Microbiological Methods, 84(2), 295-297.
22. Sogawa, K., et al. (2011). Use of the MALDI BioTyper system with MALDI-TOF mass spectrometry for rapid identification of microorganisms. Analytical and Bioanalytical Chemistry, 400(7), 1905-1911.
23. Ferroni, A., et al. (2010). MALDI-TOF mass spectrometry applications in clinical microbiology. Future Microbiology, 5(11), 1733-1754.
24. Baur, D., et al. (2010). Use of MALDI-TOF mass spectrometry for rapid identification of bacteria that are difficult to culture. Journal of Clinical Microbiology, 48(8), 3168-3175.
25. van Belkum, A., et al. (2015). Rapid clinical bacteriology and its future impact. Annals of Laboratory Medicine, 35(2), 139-144.
26. Bauer, K. A., et al. (2014). Antimicrobial stewardship programs: CAP and IDSA guidelines. Journal of Hospital Medicine, 9(8), 563-574.
27. Kumar, A., et al. (2016). Duration of hypotension before initiation of effective antimicrobialtherapy is the critical determinant of survival in human septic shock. Crit Care Med, 34(6), 1589-1596.
28. Kriegeskorte, A., Konig, A., Sander, G., Bohnert, J. A., Koenig, W., & Becker, K. (2014). Use of MALDI-TOF mass spectrometry to detect aminoglycoside resistance in Acinetobacter baumannii complex. International journal of medical microbiology, 304(5-6), 678-684. doi: 10.1016/j.ijmm.2014.04.014
29. Van Dijck, C., Vancraeynest, D., Surmont, I., & Verstraete, A. G. (2014). Rapid identification and susceptibility testing of urinary tract pathogens using MALDI-TOF MS and a modification of the standard EUCAST disk diffusion test: a pilot study. European journal of clinical microbiology & infectious diseases, 33(5), 755-762. doi: 10.1007/s10096-013-2018-3
30. Dubois,M., Buchegger, J., Brelle, S., Bonnet, R., & Girard, V. (2018). Use of MALDI-TOF mass spectrometry to identify resistance determinants in extensively drug-resistant Acinetobacter baumannii. Journal of clinical microbiology, 56(7), e00318-18. doi: 10.1128/JCM.00318-18
31. Jünemann, S., Sedlazeck, F. J., Prior, K., Albersmeier, A., John, U., Kalinowski, J., & Goesmann, A. (2013). Updating benchtop sequencing performance comparison. Nature Biotechnology, 31(4), 294-296. doi: 10.1038/nbt.2522
32. Kim, S., Kim, J. H., Park, Y. K., Park, H. J., Choi, J. Y., Kim, H. Y., & Kim, E. C. (2010). Proteomic analysis of the sarcosine-insoluble outer membrane fraction of Mycobacterium tuberculosis. Journal of proteome research, 9(12), 6721-6730. doi: 10.1021/pr100671v.
33. Singhal, N., Kumar, M., Kanaujia, P. K., & Virdi, J. S. (2015). MALDI-TOF mass spectrometry: an emerging technology for microbial identification and diagnosis. Frontiers in microbiology, 6, 791. doi: 10.3389/fmicb.2015.00791.
34. Prod'hom, G., Bizzini, A., Durussel, C., Bille, J., & Greub, G. (2010). Matrix-assisted laser desorption ionization-time of flight mass spectrometry for direct bacterial identification from positive blood culture pellets. Journal of clinical microbiology, 48(4), 1481-1483. doi: 10.1128/JCM.01780-09.
35. Kwak, Y. G., Choi, J. Y., Yoo, H. M., Lee, J. E., Kim, H. H., & Yong, D. (2011). Matrix-assisted laser desorption ionization-time of flight mass spectrometry for the rapid detection of antimicrobial resistance mechanisms and beyond. Expert review of proteomics, 8(5), 407-418. doi: 10.1586/epr.11.20

36. Otto, M. (2014). Staphylococcus aureus toxins. Current Opinion in Microbiology, 17, 32-37.
37. Lowy, F. D. (1998). Staphylococcus aureus infections. New England Journal of Medicine, 339(8), 520-532.
38. Chambers, H. F., & Deleo, F. R. (2009). Waves of resistance: Staphylococcus aureus in the antibiotic era. Nature Reviews Microbiology, 7(9), 629-641.
39. Shore, A. C., & Coleman, D. C. (2013). Staphylococcal cassette chromosome mec: recent advances and new insights. InternationalJournal of Medical Microbiology, 303(11), 685-698.
40. Cheung, A. L., & Zhang, G. (2002). Global regulation of virulence determinants in Staphylococcus aureus by the SarA protein family. Frontiers in Bioscience, 7, d1825-d1842.
41. Lindsay, J. A., & Holden, M. T. (2004). Understanding the rise of the superbug: investigation of the evolution and genomic variation of Staphylococcus aureus. Functional and Integrative Genomics, 4(4), 187-201.
42. Foster, T. J. (2005). Immune evasion by staphylococci. Nature Reviews Microbiology, 3(12), 948-958.
43. Novick, R. P. (2003). Autoinduction and signal transduction in the regulation of staphylococcal virulence. Molecular Microbiology, 48(6), 1429-1449.
44. Novick, R. P. (2003). Regulation of virulence by the staphylococcal quorum-sensing system. In Gram-Positive Pathogens (pp. 231-243). ASM Press.
45. Queck, S. Y., & Otto, M. (2011). Staphylococcus epidermidis and Staphylococcus aureus pathogenesis and immunity in humans. Annual Review of Medicine, 62, 193-206.
46. Ji, G., Beavis, R., & Novick, R. P. (1995). Bacterial interference caused by autoinducing peptide variants. Science, 267(5206), 1144-1147.
47. Otto, M. (2008). Staphylococcal biofilms. Current Topics in Microbiology and Immunology, 322, 207-228.
48. Cheung, A. L., Bayer, A. S., Zhang, G., Gresham, H., & Xiong, Y. Q. (2004). Regulation of virulence determinants in vitro and in vivo in Staphylococcus aureus. FEMS Immunology and Medical Microbiology, 40(1), 1-9.
49. Otto, M. (2009). Phenol-soluble modulins. International Journal of Medical Microbiology, 299(6), 164-169.
50. Wang, R., Braughton, K. R., Kretschmer, D., Bach, T. H. L., Queck, S. Y., Li, M., ... & DeLeo, F. R. (2007). Identification of novel cytolytic peptides as key virulence determinants for community-associated MRSA. Nature Medicine, 13(12), 1510-1514.
51. Kim, M. H., & Choi, W. C. (2020). Targeting the Agr system for the development of novel anti-virulence therapeutics against Staphylococcus aureus infections. Archives of Pharmacal Research, 43(9), 979-991.
52. Schnaith, A., Kashkar, H., Leggio, S. A., Addicks, K., Krönke, M., & Krut, O. (2007). Staphylococcus aureus subvert autophagy for induction of caspase-independent host cell death. Journal of Biological Chemistry, 282(4), 2695-2706.
53. Novick, R. P., & Geisinger, E. (2008). Quorum sensing in staphylococci. Annual Review of Genetics, 42, 541-564.
54. Nakatsuji, T., & Gallo, R. L. (2021). The role of the skin microbiota in acne pathophysiology. Journal of dermatological science, 101(2), 67-74.
55. Proksch, E., Brandner, J. M., & Jensen, J. M. (2020). The skin: an indispensable barrier. Experimental dermatology, 29(8), 501-513.
56. Gallo, R. L., & Nakatsuji, T. (2021). Microbial symbiosis with the innate immune defense system of the skin. Journal of Investigative Dermatology, 141(9), 1930-1939.
57. Schittek, B., & Kubitscheck, U. (2021). Innate immune defense of the skin: perspectives on antimicrobial peptides. Journal of Investigative Dermatology, 141(9), 1877-1889.
58. Foster, T. J. (2020). Staphylococcus. In Baron S, editor: Medical Microbiology. Galveston (TX): University of Texas Medical Branch at Galveston.
59. Foster, T. J. (2019). The Staphylococcus aureus “superbug”. Journal of Clinical Investigation, 129(7), 2526-2528.

60. Weidenmaier, C., & Peschel, A. (2020). Teichoic acids and related cell-wall glycopolymers in Gram-positive physiology and host interactions. Nature Reviews Microbiology, 18(3), 172-186.8. Inoshima, I., Inoshima, N., Wilke, G. A., Powers, M. E., & Frank, K. M. (2020). Disruption of endothelial barrier function by staphylococcal alpha-toxin: implications for pathogenesis of septic shock. Microorganisms, 8(4), 547.
61. Spaan, A. N., Surewaard, B. G., Nijland, R., & van Strijp, J. A. (2013). Neutrophils versus Staphylococcus aureus: a biological tug of war. Annual Review of Microbiology, 67, 629-650.
62. Kaplan, M. J., Radic, M., & Neutrophil extracellular traps: double-edged swords of innate immunity. Journal of Immunology, 189(6), 2689-2695.
63. Koczulla, A. R., Bals, R., & Reuter, S. (2021). The innate immune system in chronic lung disease: a comprehensive overview. Cellular and Molecular Immunology, 18(6), 1395-1410.
64. Cheng, A. G., DeDent, A. C., Schneewind, O., & Missiakas, D. (2011). A play in four acts: Staphylococcus aureus abscess formation. Trends in Microbiology, 19(5), 225-232.
65. Liang, X., Zheng, Y., & Liu, J. Q. (2020). The role of the host immune system in staphylococcal infections. Microbial Pathogenesis, 148, 104444.
66. Thammavongsa, V., Kim, H. K., & Missiakas, D. (2015). Staphylococcus aureus pathogenesis: the role of immune system evasion and pathogenicity island genes. International Journal of Medical Microbiology, 305(2), 182-188.
67. Novick, R. P., Ross, H. F., Projan, S. J., Kornblum, J., Kreiswirth, B., & Moghazeh, S. (1993). Synthesis of staphylococcal virulence factors is controlled by a regulatory RNA molecule. EMBO Journal, 12(10), 3967-3975.
68. Krakauer, T. (2019). Update on staphylococcal superantigen-induced signaling pathways and therapeutic interventions. Toxins, 11(11), 594.
69. Papageorgiou, A. C., & Acharya, K. R. (2000). Microbial superantigens: from structure to function. Trends in Microbiology, 8(3), 88-94.
70. Spaulding, A. R., Salgado-Pabón, W., & Schlievert, P. M. (2013). Models matter: the search for an effective Staphylococcus aureus vaccine. Nature Reviews Microbiology, 11(7), 507-520.
71. Liang, X., Zheng, Y., & Liu, J. Q. (2020). The role of the host immune system in staphylococcal infections. Microbial Pathogenesis, 148, 104444.
72. Li, M., & DuMont, A. L. (2019). Comparative analysis of virulence and toxin expression of global community-associated methicillin-resistant Staphylococcus aureus strains. Microorganisms, 7(7), 222.
73. Spaan, A. N., vanStrijp, J. A., & Torres, V. J. (2017). Leukocidins: staphylococcal bi-component pore-forming toxins find their receptors. Nature Reviews Microbiology, 15(7), 435-447.
74. Otto, M. (2014). Staphylococcus aureus toxins. Current Opinion in Microbiology, 17, 32-37.
75. Kohler, C., von Eiff, C., & Peters, G. (2003). Proinflammatory activities of aggregated alpha-hemolysin from Staphylococcus aureus: protection from immediate toxicity by its association with alpha2-macroglobulin. Journal of Infectious Diseases, 187(4), 536-540.
76. Richardson, A. R., Libby, S. J., Fang, F. C., & Kline, K. A. (2008). The evolution of microbial pathogenesis: the case of Candida albicans. Current Opinion in Microbiology, 11(5), 320-327.
77. Löffler, B., Hussain, M., Grundmeier, M., Brück, M., Holzinger, D., & Völker, U. (2014). Staphylococcus aureus Panton-Valentine leukocidin is a very potent cytotoxic factor for human neutrophils. PLoS One, 9(7), e100071.
78. Thammavongsa, V., Kern, J. W., Missiakas, D. M., & Schneewind, O. (2009). Staphylococcus aureus synthesizes adenosine to escape host immune responses. Journal of Experimental Medicine, 206(11), 2417-2427.
79. Nikaido, H., & Wu, J. J. (2019). Antibiotic Resistance. Advances in Experimental Medicine and Biology, 1145, 1-14.
80. Laxminarayan, R., Matsoso, P., Pant, S., Brower, C., Røttingen, J. A., Klugman, K., ... & Holmes, K. (2016). Access to effective antimicrobials: a worldwide challenge. The Lancet, 387(10014), 168-175.

81. Spellberg, B., Guidos, R., Gilbert, D., Bradley, J., Boucher, H. W., Scheld, W. M., & Bartlett, J. G. (2008). The epidemic of antibiotic-resistant infections: a call to action for the medical community from the Infectious Diseases Society of America. Clinical Infectious Diseases, 46(2), 155-164.
82. Ventola, C. L. (2015). The antibiotic resistance crisis: part 1: causes and threats. Pharmacy and Therapeutics, 40(4), 277-283.
83. Andrews, J. M. (2001). Determination of minimum inhibitory concentrations. Journal of Antimicrobial Chemotherapy, 48(Suppl. 1), 5-16.
84. Khan, G. A., Berglund, B., Khan, K. M.,Lindblad, M., & Fick, J. (2013). Occurrence and abundance of antibiotics and resistance genes in rivers, canal and near drug formulation facilities—a study in Pakistan. PLoS One, 8(6), e62712.
85. Larsson, D. G., de Pedro, C., & Paxeus, N. (2007). Effluent from drug manufactures contains extremely high levels of pharmaceuticals. Journal of Hazardous Materials, 148(3), 751-755.
86. Lowy, F. D. (1998). Staphylococcus aureus infections. New England Journal of Medicine, 339(8), 520-532.
87. Tong, S. Y., Davis, J. S., Eichenberger, E., Holland, T. L., & Fowler Jr, V. G. (2015). Staphylococcus aureus infections: epidemiology, pathophysiology, clinical manifestations, and management. Clinical Microbiology Reviews, 28(3), 603-661.
88. Otto, M. (2014). Staphylococcus aureus toxins. Current Opinion in Microbiology, 17, 32-37.
89. Deurenberg, R. H., Stobberingh, E. E., & The molecularepidemiology of MRSA. Clinical Microbiology and Infection, 10(12), 1077-1082.
90. Tong, S. Y., Davis, J. S., Eichenberger, E., Holland, T. L., & Fowler Jr, V. G.(2015). Staphylococcus aureus infections: epidemiology, pathophysiology, clinical manifestations, and management. Clinical Microbiology Reviews, 28(3), 603-661.
91. Deurenberg, R. H., Stobberingh, E. E., & The molecular epidemiology of MRSA. Clinical Microbiology and Infection, 10(12), 1077-1082.
92. Tong, S. Y., Davis, J. S., Eichenberger, E., Holland, T. L., & Fowler Jr, V. G. (2015). Staphylococcus aureus infections: epidemiology, pathophysiology, clinical manifestations, and management. Clinical Microbiology Reviews, 28(3), 603-661.
93. Deurenberg, R. H., Stobberingh, E. E., & The molecular epidemiology of MRSA. Clinical Microbiology and Infection, 10(12), 1077-1082.
94. Klevens, R. M., Morrison, M. A., Nadle, J., Petit, S., Gershman,A., Ray, S., Harrison, L. H., Lynfield, R., Dumyati, G., Townes, J. M., Craig, A. S., Zell, E. R., Fosheim, G. E., McDougal, L. K., Carey, R. B., & Fridkin, S. K. (2007). Invasive methicillin-resistant Staphylococcus aureus infections in the United States. JAMA, 298(15), 1763-1771.
95. Lowy, F. D. (1998). Staphylococcus aureus infections. New England Journal of Medicine, 339(8), 520-532.
96. Deleo, F. R., Chambers, H. F., & Musser, J. M. (2009). Community-associated methicillin-resistant Staphylococcus aureus. The Lancet, 375(9725), 1557-1568.
97. Fowler Jr, V. G., & Proctor, R. A. (2014). Where does a Staphylococcus aureus vaccine stand?. Clinical Microbiology and Infection, 20(Supplement 5), 66-75.
98. Tong, S. Y., Davis, J. S., Eichenberger, E., Holland, T. L., & Fowler Jr, V. G. (2015). Staphylococcus aureus infections: epidemiology, pathophysiology, clinical manifestations, and management. Clinical Microbiology Reviews, 28(3), 603-661.
99. Deurenberg, R. H., Stobberingh, E. E., & The molecular epidemiology of MRSA. Clinical Microbiology and Infection, 10(12), 1077-1082.
100. Mermel, L. A. (2010). Prevention of intravascular catheter-related infections. Annals of Internal Medicine, 152(5), ITC31-15.
101. Tong, S. Y., Davis, J. S., Eichenberger, E., Holland, T. L., & Fowler Jr, V. G. (2015). Staphylococcus aureus infections: epidemiology, pathophysiology, clinical manifestations, and management. Clinical Microbiology Reviews, 28(3), 603-661.
102. Deurenberg, R. H., Stobberingh, E. E., & The molecular epidemiology of MRSA. Clinical Microbiology and Infection, 10(12), 1077-1082.

103. Tong, S. Y., Davis, J. S., Eichenberger, E., Holland, T. L., & Fowler Jr, V. G. (2015). Staphylococcus aureus infections: epidemiology, pathophysiology, clinical manifestations, and management. Clinical Microbiology Reviews, 28(3), 603-661.
104. Deurenberg, R. H., Stobberingh, E. E., & The molecular epidemiology of MRSA. Clinical Microbiology and Infection, 10(12), 1077-1082.
105. Rupp, M. E., & Archer, G. L. (1994). Coagulase-negative staphylococci: pathogens associated with medical progress. Clinical Infectious Diseases, 19(2), 231-243.
106. Howden, B. P., & Grayson, M. L. (2006). Dumb and dumber—the potential waste of a useful antistaphylococcal agent: emerging fusidic acid resistance in Staphylococcus aureus. Clinical Infectious Diseases, 42(3), 394-400.
107. Davies, J., & Davies, D. (2010). Origins and evolution of antibiotic resistance. Microbiology and molecular biology reviews, 74(3), 417-433.
108. Wright, G. D. (2014). Antibiotic adjuvants: rescuing antibiotics from resistance. Trends in microbiology, 22(6), 289-291.
109. Payne, D. J., Gwynn, M. N., Holmes, D. J., & Pompliano, D. L. (2007). Drugs for bad bugs: confronting the challenges of antibacterial discovery. Nature Reviews Drug Discovery, 6(1), 29-40.
110. Silver, L. L. (2011). Challenges of antibacterial discovery. Clinical microbiology reviews, 24(1), 71-109.
111. Brown, E. D., & Wright, G. D. (2016). Antibacterial drug discovery in the resistance era. Nature, 529(7586), 336-343.
112. Bush, K., & Bradford, P. A. (2016). β-Lactams and β-lactamase inhibitors: an overview. Cold Spring Harbor perspectives in medicine, 6(8), a025247.
113. Butler, M. S., Blaskovich, M. A., & Cooper, M. A. (2013). Antibiotics in the clinical pipeline in 2013. The Journal of antibiotics, 66(10), 571-591.
114. Livermore, D. M. (2011). Discovery research: the scientific challenge of finding new antibiotics. Journal of antimicrobial chemotherapy, 66(9), 1941-1944.
115. Bhagwat, S. S., & Mundkur, L. A. (2019). Challenges and opportunities in the discovery of new antibiotics. Journal of Antibiotics, 72(1), 9-30.
116. Boucher, H. W., Talbot, G. H., Benjamin Jr, D. K., Bradley, J., Guidos, R. J., Jones, R. N., ... & Gilbert, D. (2013). 10× '20 progress—development of new drugs active against gram-negative bacilli: an update from the Infectious Diseases Society of America. Clinical Infectious Diseases, 56(12), 1685-1694.
117. Silver, L. L. (2011). Challenges of antibacterial discovery. Clinical microbiology reviews, 24(1), 71-109.
118. Bush, K., & Bradford, P. A. (2016). β-Lactams and β-lactamase inhibitors: an overview. Cold Spring Harbor perspectives in medicine, 6(8), a025247.
119. Butler,M. S., Blaskovich, M. A., & Cooper, M. A. (2013). Antibiotics in the clinical pipeline in 2013. The Journal of antibiotics, 66(10), 571-591.
120. Livermore, D. M. (2011). Discovery research: the scientific challenge of finding new antibiotics. Journal of antimicrobial chemotherapy, 66(9), 1941-1944.
121. Pasquale, T. R. (2016). Antibacterial Discovery and Development–the failure of success. Microbiology and Molecular Biology Reviews, 80(3), 943-961.
122. Spellberg, B., Bartlett, J. G., & Gilbert, D. N. (2013). The future of antibiotics and resistance. New England Journal of Medicine, 368(4), 299-302.
123. Walsh, C. (2003). Where will new antibiotics come from?. Nature Reviews Microbiology, 1(1), 65-70.
124. Butler, M. S., & Cooper, M. A. (2017). Antibiotics in the clinical pipeline at the end of 2015. The Journal of Antibiotics, 70(1), 3-24.
125. Ling, L. L., Schneider, T., Peoples, A. J., Spoering, A. L., Engels, I., Conlon, B. P., ... & Mueller, A. (2015). A new antibiotic kills pathogens without detectable resistance. Nature, 517(7535), 455-459.

126. Bugg, T. D., Wright, G. D., Dutka-Malen, S., Arthur, M., Courvalin, P., & Walsh, C. T. (1991). Molecular basis for vancomycin resistance in Enterococcus faecium BM4147: biosynthesis of a depsipeptide peptidoglycan precursor by vancomycin resistance proteins VanH and VanA. Biochemistry, 30(43), 10408-10415.
127. Melinta Therapeutics. (2017). Melinta Therapeutics announces FDA approval of Baxdela (delafloxacin) for acute bacterial skin and skin structure infections (ABSSSI).
128. Noel, G. J., Draper, M. P., Hait, H., Tanaka, S. K., Arbeit, R. D., & Hershfield, J. R. (2019). A randomized, evaluator-blind, phase 2 study comparing the safety and efficacy of delafloxacin to vancomycin plus aztreonam for the treatment of acute"Delafloxacin: A Promising Broad-Spectrum Antibiotic for Treating Bacterial Infections, Including MRSA".
129. Bassetti, M., Righi, E., & Viscoli, C. (2018). DLX, a new fluoroquinolone, for the treatment of Gram-positive and Gram-negativeinfections: a review. Future Microbiology, 13(11), 1211-1225.
130. Pullman, J., Gardovskis, J., Farley, B., Sun, E., Quintas, M., Lawrence, L., ... & Lodise, T. (2017). Efficacy and safety of delafloxacin compared with vancomycin plus aztreonam for acute bacterial skin and skin structure infections: a Phase 3, double-blind, randomized study. Journal of Antimicrobial Chemotherapy, 72(12), 3471-3480.
131. Stets, R., Popescu, M., Gonong, J. R., Mitha, I., Nseir, W., Madej, A., ... & Das, A. F. (2017). Omadacycline for acute bacterial skin and skin-structure infections. New England Journal of Medicine, 377(7), 645-653.
132. Bhagwat SS, Lahiri SD, Chopra R. Antibacterial activity of delafloxacin against contemporary Gram-positive and -negative respiratory tract pathogens isolatedfrom the United States and Europe, 2011-2012. Antimicrob Agents Chemother. 2015;59(4):2519-2528. doi:10.1128/AAC.04555-14
133. Dalhoff A. Resistance surveillance studies: a multifaceted problem—the fluoroquinolone example. Infection. 2012;40(3):239-262. doi:10.1007/s15010-012-0256-3.
134. Frieri M, Kumar K, Boutin A. Antibiotic Resistance. J Infect Public Health. 2017;10(4):369-378. doi:10.1016/j.jiph.2016.08.007
135. Hersh AL, Shapiro DJ, Pavia AT, Shah SS. Antibiotic prescribing in ambulatory pediatrics in the United States. Pediatrics. 2011;128(6):1053-1061. doi:10.1542/peds.2011-1337
136. Remy JM, Tow-Keogh CA, McConnell TS, Dalton JM, Devito JA. Activity of delafloxacin against methicillin-resistant Staphylococcus aureus: resistance selection and characterization. J Antimicrob Chemother. 2012;67(12):2814-2820. doi:10.1093/jac/dks310
137. Reddy DS, Kumar BN, Reddy GR, et al. Delafloxacin: A promising fluoroquinolone in the treatment of respiratory tract infections. J Pharmacol Pharmacother. 2017;8(2):78-83. doi:10.4103/jpp.JPP_19_17
138. Sader HS, Flamm RK, Carvalhaes CG, Castanheira M. Antimicrobial activity of delafloxacin tested against Gram-positive organisms collected from acute bacterial skin and skin structure infections in United States hospitals (2015-2016). Diagn Microbiol Infect Dis. 2018;92(1):40-44. doi:10.1016/j.diagmicrobio.2018.01.019
139. Tewari YB, Krishnamurthy S, Dholakiya RN. Delafloxacin: Anovel fluoroquinolone for the treatment of acute bacterial skin and skin structure infections. J Pharmacol Pharmacother. 2018;9(4):212-217. doi:10.4103/jpp.JPP_80_18
140. Zhanel GG, Love R, Adam H, et al. Delafloxacin: A New Fluoroquinolone with Activity against Methicillin-Resistant Staphylococcus aureus and Pseudomonas aeruginosa. Drugs. 2016;76(8):923-930. doi:10.1007/s40265-016-0571-3
141. Frieri M, Kumar K, Boutin A. Antibiotic Resistance. J Infect Public Health. 2017;10(4):369-378. doi:10.1016/j.jiph.2016.08.007
142. Dalhoff A. Resistance surveillance studies: a multifaceted problem—the fluoroquinolone example. Infection. 2012;40(3):239-262. doi:10.1007/s15010-012-0256-3.
143. Drlica K, Zhao X. DNA gyrase, topoisomerase IV, and the 4-quinolones. Microbiol Mol Biol Rev. 1997;61(3):377-392.

144. Bhagwat SS, Lahiri SD, Chopra R. Antibacterial activity of delafloxacin against contemporary Gram-positive and -negative respiratory tract pathogens isolated fromthe United States and Europe, 2011-2012. Antimicrob Agents Chemother. 2015;59(4):2519-2528. doi:10.1128/AAC.04555-14
145. Reddy DS, Kumar BN, Reddy GR, et al. Delafloxacin: A promising fluoroquinolone in the treatment of respiratory tract infections. J Pharmacol Pharmacother. 2017;8(2):78-83. doi:10.4103/jpp.JPP_19_17
146. Remy JM, Tow-Keogh CA, McConnell TS, Dalton JM, Devito JA. Activity of delafloxacin against methicillin-resistant Staphylococcus aureus: resistance selection and characterization. J Antimicrob Chemother. 2012;67(12):2814-2820. doi:10.1093/jac/dks310
147. 122. Sader HS, Flamm RK, Carvalhaes CG, Castanheira M. Antimicrobial activity of delafloxacin tested against Gram-positive organisms collected from acute bacterial skin and skin structure infections in United States hospitals (2015-2016). Diagn Microbiol Infect Dis. 2018;92(1):40-44. doi:10.1016/j.diagmicrobio.2018.01.019
148. Tewari YB,Manchanda V, Arora S. Delafloxacin: A novel fluoroquinolone for the treatment of acute bacterial skin and skin structure infections. J Pharmacol Pharmacother. 2017;8(3):120-123. doi:10.4103/jpp.JPP_26_17
149. Frieri M, Kumar K, Boutin A. Antibiotic Resistance. J Infect Public Health. 2017;10(4):369-378. doi:10.1016/j.jiph.2016.08.007
150. Dalhoff A. Resistance surveillance studies: a multifaceted problem—the fluoroquinolone example. Infection. 2012;40(3):239-262. doi:10.1007/s15010-012-0256-3.
151. Drlica K, Zhao X. DNA gyrase, topoisomerase IV, and the 4-quinolones. Microbiol Mol Biol Rev. 1997;61(3):377-392.
152. Bhagwat SS, Lahiri SD, Chopra R. Antibacterial activity of delafloxacin against contemporary Gram-positive and -negative respiratory tract pathogens isolated fromthe United States and Europe, 2011-2012. Antimicrob Agents Chemother. 2015;59(4):2519-2528. doi:10.1128/AAC.04555-14
153. Reddy DS, Kumar BN, Reddy GR, et al. Delafloxacin: A promising fluoroquinolone in the treatment of respiratory tract infections. J Pharmacol Pharmacother. 2017;8(2):78-83. doi:10.4103/jpp.JPP_19_17
154. Remy JM, Tow-Keogh CA, McConnell TS, Dalton JM, Devito JA. Activity of delafloxacin against methicillin-resistant Staphylococcus aureus: resistance selection and characterization. J Antimicrob Chemother. 2012;67(12):2814-2820. doi:10.1093/jac/dks310
155. Sader HS, Flamm RK, Carvalhaes CG, Castanheira M. Antimicrobial activity of delafloxacin tested against Gram-positive organisms collected from acute bacterial skin and skin structure infections in United States hospitals (2015-2016). Diagn Microbiol Infect Dis. 2018;92(1):40-44. doi:10.1016/j.diagmicrobio.2018.01.019
156. Tewari YB,Manchanda V, Arora S. Delafloxacin: A novel fluoroquinolone for the treatment of acute bacterial skin and skin structure infections. J Pharmacol Pharmacother. 2017;8(3):120-123. doi:10.4103/jpp.JPP_26_17.
157. Ref
158. Alexander Fleming, ‘On the Antibacterial Action of Cultures of a Penicillium, with Special Reference to Their Use in the Isolation of B. influenzæ’. Br J Exp Pathol 10 (6) (1929): 226–236.
159. Spring, D. R. ‘Chemical genetics to chemical genomics: small molecules offer big insights’. Chem Soc Rev.34 (3) (2005): 472-86.
160. Brown, D.G. and J.E. Boehm. ‘Bursting the stereotypes of rational drug design: creativity in biomolecular design’. Curr. Opin. Struct. Biol.27(2016):102-109.
161. Hughes, B. '2007 FDA Pharmaceutical Quality Oversight'. Food and Drug Administration (FDA). (2018). Retrieved 9 July 2018.
162. Kuepfer, L., and P.F. Giai. 'Virtual Screening for Bioactive Molecules: Complementing High Throughput Screening'. Altorfer, H. Rotter, J. Stables. High Throughput Screening: Methods and Protocols. Methods in Molecular Biology.1491(2017):49-64.

163. Stratton, Charles William. 'Rules of discovery: A philosophical examination of the methods of science'. History and Philosophy of Science. 19.2 (1988): 215-236.
164. Hajishengallis, G. 'Immunomicrobial pathogenesis of periodontitis: keystones, pathobionts, and host response'. Trends in immunology. 35 (1) (2014): 3–11.
165. 8.O'Neill, J. 'Tackling drug-resistant infections globally: Final report and recommendations'. Review on Antimicrobial Resistance. (2016).
166. WHO. 'WHO guidelines on use of medically important antimicrobials in food-producing animals'. (2017).
167. Spicknall, I. H., Foxman, B. and Eisenberg, J. N. S. 'A mathematical model of the evolution of antibiotic resistance and the costs of resistance in a community setting'. Epidemiol. Infect. 140 (04) (2012): 689–699.
168. Dancer, S. J. 'The role of environmental cleaning in the control of hospital acquired infection'. J Hosp Infect. 73 (4) (2009): 378–85.
169. Collignon P. 'Antibiotic use in aquaculture: benefits and impacts'. Future Microbiol. 13, (2018): 1285–1288.
170. McCarthy, M. "Educational interventions to improve antibiotic use". The Lancet Infectious Diseases. 19.2 (2019): e50-e58.
171. Lizán M, Comellas-Aragones M, de la Puente-Redondo V, Poór-Murcia L, Girona-Alarcon M. 'Strategies to reduce the use of antibiotics in animal production in the EU'. Front Vet Sci. 7, (2020): 476.
172. Marshall BM, Levy SB. 'Food animals and antimicrobials: Impacts on human health'. Clin Microbiol Rev. 24 (4) (2011): 718–33.
173. A uld, D. S. and Kornberg, A. 'Isolation and properties of DNA gyrase from Escherichia coli'. Proc Natl Acad Sci U S A.74(11) (1977): 5462–5466.
174. Drlica, K. and J. Zhao, X. 'DNA gyrase, topoisomerase IV, and the 4-quinolones'. Microbiol. Mol. Biol. Rev. 61 (3) (1997): 377–392.
175. Hooper, D. C. 'Quinolones'. In: Antibacterial Chemoterhapy, 4th ed., Hooper, D. C., Rubinstein, E. (Eds). ASM Press, Washington, D.C. (1999): 307-330.
176. Guerrant, R. L. and Moore, S. R. 'Challenges in Global Public Health: Globalization and Infectious Diseases—A Tropical Perspective'. Emerg Infect Dis. 10 (9) (2004): 1671–1672.
177. Hein, H. H., Moody, J., and Jakobi, K. 'Differentiation of bacterial species with the aid of mass spectrometry'. Rapid Commun Mass Spectrom. 8 (2) (1994): 137–140.
178. 21.Kapoor, G., Saigal, S., & Elongavan, A. 'Action and resistance mechanisms of antibiotics: A guide for clinicians'. J Anaesthesiol Clin Pharmacol. 33(3), (2017): 300-305.
179. Nina Kumarasamy, Mark Toleman, Tim Walsh, laurent Poirel, and Patrice Nordmann. 'Emergence of a new antibiotic resistance mechanism in India, Pakistan, and the UK: a molecular, biological, and epidemiological study'. The Lancet Infectious Diseases. 10 (9)(2010): 597-602.
180. Fischbach, M. A. and Clardy, J. 'One Pathway to Many Drugs'. Chem. Biol. 14 (2) (2007): 105–106.
181. Ng, Manjula, et al. "Antibiotic susceptibility patterns of ESβL producers isolated from the mobile phones." *Evidence-Based Complementary and Alternative Medicine* 2022 (2022).
182. Muñoz, K. A. and Olivera, B. M. 'Platinum binding to proteins: cysteine accessibility and sulfenic acid reactivity in calmodulin'. Chem Res Toxicol. 31 (1) (2018): 24-31.
183. Marchese, A., E.S. Boissonnas, A. Casrenti, M. Kubin, and J. Rossi. 'New antibiotic adjuvants: Myth or reality?'. J Glob Antimicrob Resist. 19 (2019): 222-225.
184. Wright G. D. 'The antibiotic resistome: the nexus of chemical and genetic diversity'. Nat Rev Microbiol. 5(3) (2007): 175-86.
185. Alghamdi, Saad. "The role of vaccines in combating antimicrobial resistance (AMR) bacteria." *Saudi Journal of Biological Sciences* 28.12 (2021): 7505-7510.
186. Aeschlimann, J R. 'The role of multidrug efflux pumps in the antibiotic resistance of Pseudomonas aeruginosa and other Gram-negative bacteria'. Insights from the Society for Applied Microbiology. 2 (2003): 7-12

187. Atwah, Banan, et al. "Susceptibility of Diabetic Patients to COVID-19 Infections: Clinico-Hematological and Complications Analysis." *Vaccines* 11.3 (2023): 561.
188. Courvalin, P. 'Vancomycin resistance in gram-positive cocci'. Clin Infect Dis. 42 Suppl.1(2006): S25-34.
189. Munita, J. M. and Arias, C. A. 'Mechanisms of Antibiotic Resistance'. Microbiol Spectr. 4.2(2016): 4: (2016).
190. Katz, Leonard, and Richard H. Baltz. "Natural product discovery: past, present, and future." *Journal of Industrial Microbiology and Biotechnology* 43.2-3 (2016): 155-176.
191. Cobb, Ryan E., et al. "Drug discovery and development via synthetic biology." *Synthetic biology*. Academic Press, 2013. 183-206.
192. Ayon, Navid J. "High-Throughput Screening of Natural Product and Synthetic Molecule Libraries for Antibacterial Drug Discovery." *Metabolites* 13.5 (2023): 625.