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Journal of Microbes in Health and Disease

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Review

Staphylococcus aureus—A Quiet Human Body Resident and Sometimes a Feared Enemy

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How To Cite: Lazar, V.; Badaluta, V.-A.; Apetrei, R.M.; et al. Staphylococcus Aureus—A Quiet Human Body Resident and Sometimes a Feared Enemy. *Journal of Microbes in Health and Disease* **2025**, *1*(1), 100002.

Received: 26 November 2024
Revised: 13 February 2025
Accepted: 13 May 2025
Published: 15 May 2025

Abstract: *Staphylococcus aureus (S. aureus)* is one of the most common pathogens and likely an ancient commensal component of human microbiota. From a global perspective, *S. aureus* ranks among the most frequent pathogens affecting humans and animals, with significant morbidity and mortality rates. It is a commensal bacterial species present in approximately 30% of individuals, residing on the skin and mucosal surfaces. However, it is also the etiological agent of a wide range of infections, including pyogenic skin infections, superficial infections, deep tissue, and organ infections. These can progress to systemic conditions such as bacteremia and severe sepsis. Additionally, *S. aureus* produces enterotoxins that can lead to food poisoning. This highly versatile opportunistic pathogen is implicated in device-related infections associated with biofilm formation, as well as toxin-mediated conditions such as scalded skin syndrome and toxic shock syndrome (TSS), which are mediated by exfoliatins and TSST-1 (toxic shock syndrome toxin-1), respectively. *S. aureus* possesses a wide array of virulence factors, including toxins with superantigen properties that can trigger a "cytokine storm" and hyperinflammation. The incidence of *S. aureus* infections has risen over the past two decades, including both community-acquired infections—such as skin and soft tissue infections caused by virulent, β-lactam-resistant strains—and hospital-acquired infections, including infective endocarditis and prosthetic device-related infections. This increase in incidence has also been marked by the rise of antibiotic-resistant strains, particularly methicillin-resistant *S. aureus* (MRSA) and, more recently, vancomycin-resistant clones. In this review, we aim to highlight the distinctive characteristics of *S. aureus*, a ubiquitous bacterial species and opportunistic pathogen that straddles the line between its commensal role and its aggressive profile as an etiological agent of numerous infections. This dual nature is due to its genetic adaptability, which enables resistance to various environmental presure and antimicrobial agents. The tolerance of biofilm-encased cells to antibiotics, its extensive repertoire of virulence factors, and its remarkable fitness. The increasing prevalence of immunocompromised individuals has further contributed to its pathogenic potential. Finally, we summarize the primary alternative and complementary anti-infective strategies for addressing *S. aureus* infections.

Keywords: *Staphylococcuus aureus*; opportunistic pathogen; virulence factors; biofilm tolerance; biofilm associated infections; alternative antiinfectious strategies

1. Introduction

Staphylococcus aureus (S. aureus) is among the most common pathogens and probably has been a commensal component of human microbiota for millennia [1]. From a global perspective, *S. aureus* actually ranks between the most frequent pathogens in human and animals, with great morbidity and mortality indices, proved by the multitude of scientific papers, posted on the PubMed website in the last 5 years: there are 41.724 articles about *S. aureus*, 26.155 articles about *S. aureus* infections and 11.155 articles about MRSA strains [2].

S. aureus is a commensal bacterial species present in approximately 30% of people, residing on the skin and mucosal surfaces, including the armpits, groin, and perineum [3,4]. It is also the etiological agent of a wide range of infections, ranging from pyogenic skin and superficial infections—such as impetigo, folliculitis, furuncle, carbuncle, and abscesses—to deep tissue infections, including endocarditis, septic arthritis, osteomyelitis, pleuropulmonary infections, urinary tract infections, deep organ abscesses, and thrombophlebitis, which can potentially evolve into systemic infections, such as bacteremia and severe sepsis [5]. This opportunistic pathogen is also implicated in device-related infections due to biofilm formation and toxin-mediated infections, such as scalded skin syndrome (mediated by exfoliatins) and toxic shock syndrome (TSS) (mediated by TSST-1/TSS toxin-1). Additionally, it is often associated with food poisoning caused by thermostable enterotoxins produced by *S. aureus* in contaminated food, which can be accidentally ingested [6].

The incidence of *S. aureus* infections has increased over the past two decades, including both community-acquired infections (such as skin and soft tissue infections caused by virulent strains resistant to β-lactams) and hospital-acquired infections (such as infective endocarditis and prosthetic device infections) [7]. This increased incidence has also been accompanied by a rise in antibiotic-resistance, primarily MRSA strains and, more recently, vancomycin-resistant clones [4,8]. Since the first VRSA isolate was identified in a diabetic pacient, Michigan-USA, in 2002, a total of 52 VRSA strains have been reported globally [9,10].

The clinical picture of MRSA carriage has different shapes, from asymptomatic colonization of the nasal mucosa to moderate skin and soft tissue infections, to invasive infections with fulminant evolution and with high mortality [11]. There are studies that have proven that MRSA strains are resistant to most β-lactam antibiotics, but also to a wide range of other antimicrobial substances, so that infections are difficult to manage and the treatment is very expensive [12].

Although the previous options for MRSA infections' therapy are limited, now there are under development several new antimicrobials. In addition, an efficient combating of these infections will also be possible through immunoprophylaxis, several vaccine candidates being now under development [13,14].

A comprehensive analysis, based on 471 million records of infection cases (from 204 countries/territories), using statistical methods has estimated that in 2019 a number of 4.95 million deaths were correlated with bacterial antimicrobial resistance (AMR); among these fatal cases, the lower respiratory infections were the cause for more than 1.5 million of deaths related with AMR and 1.27 million of deaths were directly due to AMR strains [15]. Between the etiological agents, the MRSA strains caused more than 100,000 deaths in the same year and other pathogens caused each between 50,000–100,000 deaths. In 2019, the leading pathogens counting for the global AMR were: *Escherichia coli*, *S. aureus*, *Klebsiella pneumoniae*, *Streptococcus pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, all being opportunistic pathogens and identified by World Health Organization (WHO) as health priority, underlining the necessity of a concerted, global combat plan [16]. Another worrying fact is that a committee of experts (Eurosurveillence program—European Centre for Disease Prevention and Control (ECDC)) estimated that until the year 2050, the number of fatal cases due to bacterial infections with resistant strains will reach about 10 million people annually in the world, exceeding the cancer mortality [17]. The major concern regarding these opportunistic pathogens is their great adaptability, correlated with many virulence factors, resistance mechanisms and fitness. Therefore, the combat plan must include surveillance along with intensified research on new strategies for preventing or managing infections caused by multidrug-resistant (MDR) and virulent strains. These strategies should encompass the development of new anti-infective and anti-pathogenic drugs, including antibiotics, alternative or complementary antimicrobials, and also new vaccines [14,18].

In this review, our aim was to highlight the special hallmarks of *Staphylococcus aureus*, an ubiquitous bacterial species and opportunistic pathogen, balancing between the commensal, friendly status and the aggresive profile and an etiological agent of a lot of infections due to its high level of genetic resistance and tolerance of biofilm encased cells to antibiotics, to its numerous virulence factors and fitness level and all this against the

backdrop of the increasing number of immunodeficient persons. In the same time, we summarized the main alternative or complementary antiinfectious strategies addressed to *S. aureus* infections.

2. Discussion

2.1. Short Characterization of S. aureus

S. aureus is a member of the human and mammals' microbiota; it is omnipresent in soil and dust and even is a non-sporogenous bacteria, it is very resistant in external environment, to dryness conditions and UV radiations [19], due to its famous golden pigment—staphyloxantin (STX) which also inspired the name of the species.

Normally, the commensal behavior of *S. aureus* strains, persists as long as the cutaneous and mucosal barriers are intact. However, if these bariers are damaged, either accidentally or by surgical interventions, *S. aureus* can easily penetrate through these gaps, reaching the underlying tissues or even the bloodstream, leading to an infectious process [14]. Normally, *S. aureus* cells are nonpathogenic and act as commensals on the skin and mucosa.

The adhesins and the specific coagulase protein of *S. aureus* protect the staphylococci against host cellular and humoral immune effectors. All of these factors contribute to the transition of a normal microbiota member into an opportunistic pathogen, or "pathobiont," capable of causing superficial infections of the skin and soft tissues, particularly during childhood and with increased incidence in older age [8]. Furthermore, it can also lead to invasive and chronic infections, especially in immunocompromised hosts [18].

S. aureus is considered a model organism for Gram-positive bacteria and a member of the ESKAPE group (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* spp.), being a common opportunistic pathogen. *S. aureus* strains, as well all these ESKAPE members, are also biofilm formers and hospital-acquiered (nosocomial) pathogens [20].

Bacterial colonization of the chronic wounds delays their healing by several mechanisms: bacterial invasion, expression of virulence traits that amplifies the bacterial pathogenicity (e.g., by production of secreted virulence factors, development of mono- or polymicrobial biofilms), genetic resistance and/ or adaptive resistance/tolerance to antimicrobial agents, and ability to destroy or avoid the host immune effectors. Moreover, bacterial synergism in polymicrobial wound infections (e.g., *S. aureus* and *P. aeruginosa*) increases the virulence and persistence of infection, but also a decreased response to therapy and chronic, recidivant infections [21,22]. Moreover, bacterial synergism in polymicrobial wound infections (e.g., *S. aureus* and *P. aeruginosa*) increases the virulence and persistence of infection, but also a decreased response to therapy and chronic, recidivant infections. In chronic venous ulcers and other chronic skin lesions allowed *S. aureus* and *P. aeruginosa* were most frequently isolates [23].

S. aureus is the most frequent species isolated from chronic wounds, including diabetes foot infections [12] and it is well known that MRSA clones cause severe and difficult to treat infections. The strains persist in the hospital environment, where, under the selective pressure of antibiotics, they evolve, with the expression of β-lactamase coding genes, as well as some metabolic and virulence genes. Moreover, they can transfer resistance genes to other species directly or indirectly. Besides the high level of antibiotic resistance, the pathogenicity of *S. aureus* also depends on the virulence profile of different clones (exoenzymes, exotoxins, staphylococcal superantigens, as well as bacterial adhesins and biofilm formation) [23,24].

2.2. Virulence and Pathogenicity

S. aureus cells have a commensal behavior if is present on intact skin and mucosa. But if these natural barriers are damaged, either accidentally or post-surgical interventions, *S. aureus* can pass through these gaps and reach the inner tissues or even the bloodstream, leading to an infectious process.

The bacterial virulence is a multifactorial, dynamic property, influenced by the clinical context, respectively host and pathogen condition, at the moment of infection [14]. Several studies are foccused on AMR profile of pathogens, and only a few on the correlation between virulence profile of bacteria isolated from different chronic infections and host immune response, with influence on the clinical outcome [25,26]. In addition to AMR, the commensal *S. aureus* can become pathogenic, causing a wide range of diseases, from mild to serious, life-threatening conditions.

S. aureus is armed with a plethora of virulence factors, including aggressins, adhesines, invasines, toxins (Table 1).

Table 1. *S. aureus* virulence factors involved in the pathogenesis of skin infections.

	The Involved Virulence Factors	**Biological Activity**	**References**
Step 1 Establishment of the infection outbreak	Leukocidin	Destroy phagocytes after epithelial breach and systemic invasion	[27,28]
	Capsular polysaccharide 5 (CP5) or 8 (CP8)	Inhibit phagocytosis by hiding the antigenic proteins at the surface of the bacterial cell	[27,29]
	Exopolysaccharide PIA (polysaccharide intercellular adhesin)	Stimulate the cell adherence and generate persistent biofilms	[27,30]
	Staphyloxanthin	Protects *S. aureus* cells from ROS (hydrogen peroxide and hydroxyl radicals)	[27,31]
Step 2 Tissue adherence and invasion	**The involved virulence factors**	**Biological activity**	
2.1. Adherence	MSCRAMM family (microbial surface components recognizing adhesive matrix molecules): protein A, clumping factors A and B, and the fibronectin-binding proteins	Surface-anchored proteins that mediate the attachment to the endothelium during endovascular infections, and adhesion to fibronectin present in the extracellular matrix of the tissues	[32,33]
	Secretable Expanded Repertoire Adhesive Molecules (SERAMs): extracellular adherence protein (Eap), the extracellular matrix binding protein (Emp), and the extracellular fibrinogen-binding protein (Efb)	Facilitate the attachment of *S. aureus* to eukaryotic cells, platelets, extracellular matrix proteins	[34]
	Collagen-binding protein Cna	Bind to sites in which collagen fibers are cleaved, such as in wounded, injured, or inflamed tissues	[35]
	Amyloid-forming—proteins (SasG)	Responsible for *S. aureus* squamous epithelium adhesion	[36]
	Fibronectin binding proteins (FnBPs)	human corneocyte adherence	[37]
2.2. Invasion	Hemolysins	Erythrocytes lysis by creating cell membranes pores or dissolving cell wall components	[38]
	Panton-Valentine Leukocidin (PVL)	Pore-forming toxins that kill immune cells (phagocytes, natural killer cells, dendritic cells, and T lymphocytes) and erythrocytes	[28]
	Exfoliative Toxins (ETs): ETA, ETB, ETC, ETD, ETE	Serine proteases that cause epidermal dissociation of the human skin	[39]
	Epidermal Cell Differentiation Inhibitor (EDIN A, B and C) Exotoxins and EDIN like exotoxins	Compromise the integrity of the endothelium barrier, promoting bacterial colonization	[27]
Step 3 Infection persistence	**The involved virulence factors**	**Biological activity**	**References**
3.1. Abscess formation	Staphylocoagulase Coa and von Willebrand factor-binding protein (vWbp)	produce fibrin clots by nonproteolytically activation of prothrombin, in order to inhibit leukocyte infiltration.	[40,41]
	Protein A	pro-inflammatory function for proper skin abscess formation but also for healing	[40,42]

	Phenol-Soluble Modulins (PSMs)	damage keratinocytes and induce skin inflammation	[40,43]
	Clumping factor B	binds to loricrin, important step in early abscess formation.	[40,44]
3.2 Chronicity correlated with biofilm development	MSCRAMM family	biofilm formation and immune evasion	[33,40,45]
	Polysaccharide intercellular adhesin (PIA, also called PNAG for poly-N-acetyl glucosamine)	major component of the staphylococcal biofilm matrix that prevents phagocytosis	[40]
	Extracellular DNA (eDNA)	protect the mature biofilm cells from deleterious environmental factors (antibiotics and the host immune effectors)	[40,46]
	Teichoic acids	Essential in the first step of biofilm formation, with higher content of wall teichoic acids for Vancomycin-intermediate *S. aureus* strains	[40,47]
3.3 Internalization, persistence, and dissemination	Fibronectin binding proteins (FnBPs) and extracellular adherence protein (Eap)	Invasion of non-phagocytic cells by binding the cell surface fibronectin and phagosome escape	[40]
	Degradative exoenzymes (proteases)	Induce the detachment of the biofilm cells clusters and dissemination	[40,48]
	Staphylokinase	Promote dissemination and immune evasion by activation of fibrinolysis	[40,49]

Its virulence pattern is influenced by the unique combination of toxins and immunomodulatory gene products, which may differ by geographic location and source of isolates: healthcare-associated infections (HAIs) and community-acquired infections (CAIs). The prevalence of MRSA strains can be mostly CAI instead of HAI [50]. It is necessary the precise identification of MRSA clones for the decolonization procedure [51], to allow the appropriate treatment, thus avoiding their epidemic hospital and community spreading, and also the risk of new clone appearance [50].

Establishment of the infection outbreak. *S. aureus* produces coagulases to polymerize fibrin and form an encapsulated abscess around the infection site. The bactericidal capacity of polymorphonuclear neutrophils (PMNs), which are present in high numbers within an abscess, is reduced by leukocidins and other virulence factors that interfere with the opsonophagocytosis and killing property of PMNs [52]. *S. aureus* can compromise the antibodies opsonization process by using a polysaccharide microcapsule and surface proteins such as SpA (*Staphylococcus* protein A) and Sbi (Ig-binding protein), which have the ability to bind IgG via Fc domain, rather than in the conventional manner [53].

The bacteria can also inhibit the complement system activation by several small secreted inhibitory factors. Phagocytosed bacteria can survive within the PMNs by producing inhibitory enzymes of ROS (reactive oxygen species), such as catalase, superoxide dismutase (SodA), peroxidase inhibitor (SPIN), staphyloxanthin (STX—inhibiting the bactericidal oxidative burst in PMNs and production of bactericidal oxygen-dependent factors) [54], and extracellular adherence protein (Eap) (inhibiting elastase) [5].

Other virulence factors of *S. aureus* strains produce cell wall modifications by multiple peptide resistance factor (MprF), D-alanine transfer proteins (DltA, DltB, DltC and DltD) which protect bacteria against defensins. *S. aureus* cells also produce cytolytic toxins that kill PMNs. Besides these cytotoxins, *S. aureus* secretes leukocidins which are pore-forming proteins (α-toxin and several two-component leukocidins, such as Panton–Valentine leukocidin (PVL) [54] and small peptide (phenol-soluble modulin (PSM) peptides) toxins [55]. Staphylokinase (SAK) also cleaves IgG1 and IgG3 and C3b (opsonins), inhibiting the opsonophagocytosis [56].

SAK is a staphylococcal fibrinolysin, secreted in late exponential phase and positively regulated by the *agr* gene regulator [57]. SAK activates plasminogen to form plasmin, which digests fibrin clots. As a result, the fibrin layer formed to isolate and protect the bacterial cells, is degraded by the action of SAK, releasing live cells and pus. The binding of SAK to plasminogen induce conformational changes that lead to its conversion into plasmin, a proteolytic enzyme involved in bacterial invasion [58]. In plasma in the absence of fibrin, the plasmin-SAK complex is neutralized by α_2- antiplasmin, resulting in lysis; however, in the presence of fibrin, the inhibition is delayed [59].

The staphylococcal toxins with special properties include the so-called superantigen toxins, such as toxic shock syndrome toxin-1 (TSST-1), enterotoxin type A (SEA), staphylococcal enterotoxin-like X (SEIX) and a few others. These toxins are classified as superantigens, because they can directly bind to major histocompatibility

complex molecules (MHC class II) on antigen-presenting cells (APCs), resulting in polyclonal activation of T lymphocytes and an increased release of pro-inflammatory cytokines. This leads to severe systemic inflammation or hypercytokinemia, also known as *cytokine storms*. This process contributes to hyperinflammation through polyclonal, non-specific activation of T lymphocytes. Like other exoproteins and cell surface virulence factors, staphylococcal superantigens are regulated by complex mechanisms, including global regulators such as *agr*, *sae*, *rot*, and *srr* [60].

Adherence. Infections caused by *S. aureus* involve bacterial adhesion to the host extracellular matrix after crossing the skin barrier [61]. Its multiple adhesins belong to two groups: one is mostly represented cell wall-anchored proteins, known as cell surface-associated molecules designated as MSCRAMMs (Microbial Surface Components Recognizing Adhesive Matrix Molecules)—represented by protein A, collagen-binding protein, elastin-binding protein, fibronectin, and soluble, secreted molecules called SERAMs (Secretable Expanded Repertoire Adhesive Molecules), represented by fibrinogen binding protein, Eap (extracellular adhesin protein) and Emp (extracellular matrix binding protein) and coagulase—the species specific enzyme [5,61].

S. aureus surface proteins (for example, fibronectin-binding protein A (FnBPA), FnBPB, clumping factor A (ClfA), ClfB and collagen adhesin (Cna)) bind to extracellular matrix proteins and enable the bacteria to attach to and multiply on wounded tissues (Figure 1) [55]. The capacity of *S. aureus* to adhere to and form biofilms on cellular and inert sufaces such as medical devices of plastic or metal, makes from *S. aureus* a frequent agent of biofilm-associated infections (formed on different catheters, implants, assisted breathing ventilator etc.). The subsequent influx of polymorphonuclear leukocytes (PMNs), attracted by *S. aureus* cells determines the local inflammation [62].

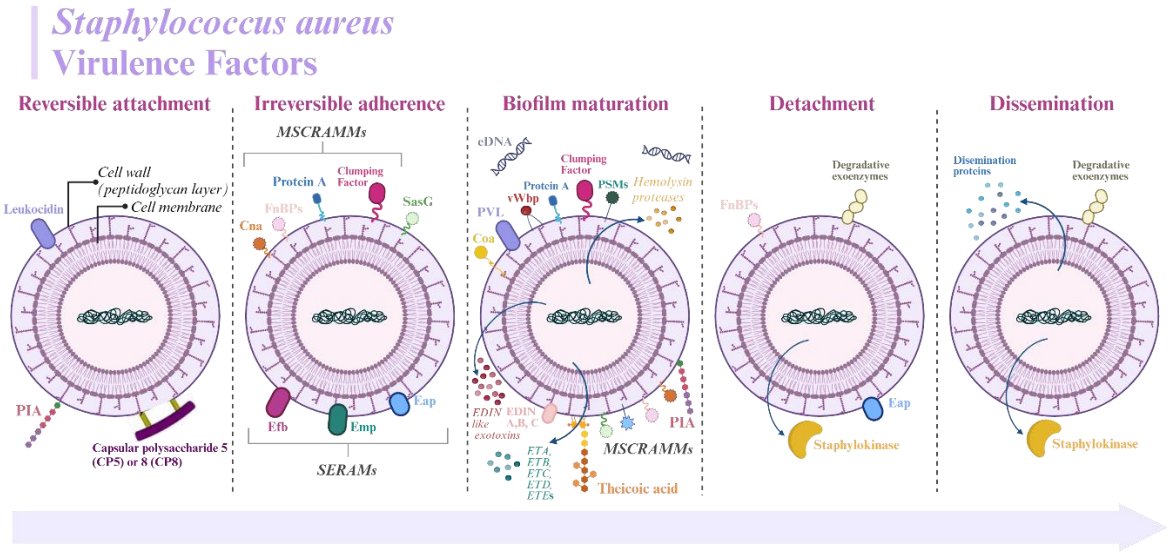

Figure 1. *Staphylococcus aureus*, the virulence factors correlated with biofilm development (original image created with www.BioRender.com, accessed on 28 January 2025).

Abscess formation. A main role in *S. aureus* viulence and pathogenicity is played by coagulase proteins that cause the transformation of the fibrinogen in fibrin and formation of a pseudo-capsule surrounding contaminant bacteria and also infiltrated PMNs. In this way, it is stopped the further leukocyte influx and the contact with the humoral immune effects [63]. So, *S. aureus* can prevent opsonization; the effect is also due to production of a polysaccharide microcapsule and inhibition of the complement cascade [64]. But, it was observed that microcapsule is absent from important MRSA clones such as USA300 [65]; however, this clone has an epidemiological risk, being easily transmitted in community and causing USA300 CA-MRSA infections [66].

Bacteria that are ingested by PMNs can survive, by counteracting PMNs killing mechanisms [54], but also by gradually destroying them by production of cytolytic toxins. For example, many CA-MRSA clones produce pore-forming peptides—PSMs and protein toxins (α-haemolysin) and several bi-component leukocidins such as the PVL, which bind to leukocyte membranes, leading to pore formation and subsequent lytic cell death [65,67], thereby increasing bacterial virulence.

The infections with *S. aureus* determine an acute inflammation due to activated and necrotic PMNs. The strong stimulation of Toll-like receptor 2 (TLR2) of PMNs depends on an intense production of PSM peptides, as

response to the virulence activator Agr. PSMs are involved in mobilization of lipoproteins from the bacterial cytoplasmic membrane, which are TLR2 agonists. Thus, this essential role of TLR2 dependens on agonist release by bacterial surfactants; it is proved that TLR 2 is involved in acute inflammation and sepsis [62]. The inflammation is further amplified by *S. aureus* specific toxins with the quality of superantigens (TSST-1, enterotoxins) [60].

Abscesses can release live bacteria to the surface of the skin and/or the bloodstream at later stages; the plasminogen-activating protein SAK might contribute to abscess rupture and bacterial dissemination [14].

Systemic infection. Abscess rupture, at later stages, can be provoked by a mechanical pressure, but also due to a non-enzymatic exoprotein with the oposite effect to coagulase, named fibrinolysin or staphylokinase (SAK), produced in late exponential phase, when the nutrients are becoming scarce. As it was shown the binding of SAK to plasminogen determines its sterical changes and its conversion to plasmin, a proteolytic enzyme of broad-spectrum that facilitates bacterial invasion [58]. SAK is a produced by a majority of *S. aureus* strains [59]. Due to SAK action, the protective layer of fibrin is thinner, more friable and when it breaks it will release its content—pus and live bacteria, outside, on the skin surface (being easily disseminated), or inside the body, in the blood circulation, causing bacteraemia. Endovascular *S. aureus* can adhere to endothelial surfaces and platelets [68], and this adhesion can initiate endocarditis, promote the formation of metastatic abscesses or induce bacterial uptake into endothelial cells, where the bacteria are difficult to reach by antibiotics and host defense molecules [67]. The agglutinating activity of coagulases is thought to contribute to systemic blood coagulation, and massive release of Microorganism-Associated Molecular Pattern (MAMP) molecules along with superantigen toxins able tu induce cytokine storms, leading to fulminant systemic inflammation, sepsis and multi-organ failure (MOF), if the endovascular dissemination of the bacteria cannot be stopped [14,69].

Researchers found that MSSA strains commonly produce polysaccharide intercellular adhesin (PIA)-dependent biofilms. On the contrary, the release of eDNA (extracellular DNA) and the expression of a number of sortase-anchored surface proteins are implicated in the biofilm phenotype of MRSA strains [4,70–72]. Nuc1 and Nuc2 nucleases produced by *S. aureus* modulate biofilm formation and immune evasion. Unlike nuc1, which is proved to contribute to immune evasion, nuc2 is not able to degrade neutrophil extracellular traps [71].

The severe evolution of *S. aureus* infections and the risk of bacteraemia-associated mortality depends on bacterial strains virulence/resistance, but also on host factors, such as age and comorbidities. The role of the bacteria in infections severity is less understood, because it is complicated by the multifactorial nature of staphylococcal virulence, which has so far delayed a robust mapping between genotype, phenotype and infection outcome [73].

2.3. Virulence Genes Regulation and Adaptation

Bacteria have complex signal communication networks and the ability to integrate these intercellular signals and give a response according to environmental conditions by regulation of gene expression (activation or repression); this ability is essential for survival both outside and inside a host. Bacterial cells communication is mediated by small, diffusible signal molecules called autoinducers (AIs). The communication between Gram positive bacteria is mediated by molecules belonging to AIs I type (peptidic molecules or AIPs) and to AIs 2 type (furanones), which mediate the universal bacterial language. The concentration of these AIs is dependent on cellular density of a bacterial community and is sensed by all component cells by Quorum Sensing (QS) mechanism. As cell density increases, the concentration of these molecules will also increase and over a critical point, these self-produced signals will modify the gene expression (including virulence genes) through protein regulators' intervention and the community' behavior will be adapted to new conditions [73,74]. This type of gene regulation is named *quorum* sensing (QS) and response (meaning an adequate response in a cell density-dependent manner) [75].

Virulence is a property with influence on host-pathogen interaction and coevolution. Even the AMR genes are not real virulence genes, they constitute a survival advantage for pathogens and amplify the virulence and fitness of MDR strains, representing at present a great challenge for the medical field [20,76].

The expression of *S. aureus* virulence factors is regulated by the quorum-sensing system *agr*. Considering that a high number of *S. aureus* strains are more difficult to be treated due to their resistance to antibiotics and virulence factors, the elucidation of *S. aureus* pathogenesis at molecular level becomes imperative in the fight against this major human pathogen, in order to find new therapeutic strategies [77].

Many virulence genes are found on mobile genetic elements; thus, their combination differs substantially between clones and even between closely related strains. The correlation between potential association of specific virulence factors in different strains of *S. aureus* with certain types or aggressiveness and the various forms of

pathologies remains elusive, probably because many of these factors have redundant, partially overlapping functions. Furthermore, many virulence factors cannot be investigated in animal models because they are human-specific [73].

Most of the *S. aureus* virulence factors are regulated by the accessory gene regulator (Agr) quorum-sensing system and other regulatory systems [78]. Many CA-MRSA clones, including USA300, have very active Agr systems, leading to an intense expression of toxins and having a high capacity to produce SSTIs (skin and sot tissue infections) and invasive infections, even in healthy individuals [79]. Contrary, many HA-MRSA clones contain an additional cassette—SCC*mec*, coding for a phenol-soluble modulin (PSM*mec*), whose mRNA diminish Agr expression [80]. By consequence, Agr system is not very active in many HA-MRSA clones, which express lower amounts of toxins, but higher levels of adhesins. These clones often cause bacteraemia, by infected catheters or implanted medical devices. Probabably, a high virulence could be detrimental for *S. aureus* strains in bacteraemia, since many isolates from systemic infections proved to carry Agr-inactivating point mutations [81]. Elucidating virulence mechanisms whose inhibition would render *S. aureus* most vulnerable will be crucial for the development of new preventive and therapeutic strategies against MRSA [14].

Mobility is crucial for microorganisms/ bacteria to avoid dangers and find nutrients in the environment, important to their survival, but also for virulence expression and pathogenicity [82]. Recent studies have demonstrated that nonmotile bacteria are able to attach to the motile ones and to reach favorable places [83]. The studies proved that *S. aureus*, despite being nonmotile coccoid bacterium, is capable to moving and spreading on a soft agar surface. This ability is mediated by the accumulation of water whitin the cell community of a colony and synthesis of biosurfactants called phenol soluble modulins (PSMs) [84], which reduce the water's surface tension and enable the cocci to spread. In an ecosystem, *S. aureus* interacts with other bacteria and microorganisms whitin a community. For instance, *S. aureus* and *P. aeruginosa* are two human pathogens that often share the same niche [20]. It has been demonstrated that swimming *P. aeruginosa* promotes the dispersion of *S. aureus* [83]. A recent study revealed that *S. aureus* can hitchhike on *P. aeruginosa* via interactions between the teichoic acids in the *S. aureus* cell wall and lipopolysaccharides of *P. aeruginosa*. The hitchhiking motility of *S. aureus* facilitated by *P. aeruginosa* has also been observed in vivo, using both a *Caenorhabditis elegans* model and a mouse model [85]. Thus, it has been established that even nonmotile microorganisms in multispecies communites can often employ a"hitchhiking" strategy to move, enabling them to overcome environmental stresses and position themselves in niches favorable for their survival.

2.4. Resistance to Antibiotics

AMR is a top priority problem for public health and an increasing phenomenon which causes great concern. AMR is a relativelly recent clinical threat, evolved during the last eight decades, after the industrial production and clinical availability of penicillin fallowed by other antibiotics which were the cause of the first wave of resistance [86] and due to the intensive use of these miraculous drugs [87]. Thus, a natural and ancient evolutionary phenomenon—antibiosis, excessively exploited, accelerated the occurrence of resistance, under the selective pressure of therapeutical antibiotics. Even if they have made a major contribution to reducing of global morbidity and mortality rates by infectious diseases, the price was an unexpected, paradoxical, adverse effect, namely a progressively increased AMR [87].

S. aureus, especially the antibiotic-resistant strains determine a high morbidity and mortality indices of HAI caused by septic shock and severe sepsis. By comparison to Methicillin-susceptible *S. aureus* (MSSA), the infections produced by MRSA led to higher mortality, morbidity, accompanied by economic loss, being heavy burden on healthcare around the world [88]. Regardless, *S. aureus*, both MRSA and MSSA, will remain one of the main human and animal pathogen [89]. During the latest several decades, the AMR evolved due to the long-term and misuse (sometimes unnecessary) of antibiotics, currently driving to the frequent unsuccessful treatments with available antibiotics. If antibiotics act by inhibiting bacterial growth or cellular viability and as selective pressure factors inducing resistance, the anti-pathogenic strategies targeting bacterial virulence genes expresion might be less possible to develop drug resistance [14].

Since the 1960s, MRSA strains have emerged—just one year after the clinical introduction of methicillin, which was developed to combat penicillin-resistant *S. aureus* strains. These strains have since achieved global dissemination, becoming a leading cause of both HAIs and CAIs. This marked the second wave of antibiotic resistance [89]. A significant geographical variation in the MRSA burden has been observed, largely due to differences in infection control measures and the pathogen-specific characteristics of circulating clones.

MRSA clones have arisen from susceptible strains through the independent acquisition of the staphylococcal cassette chromosome mec (SCC*mec*), which contains genes encoding proteins that confer resistance to most β-

lactam antibiotics, including methicillin [90]. The success of MRSA is further ensured by the extensive arsenal of virulence factors produced by *S. aureus* strains, combined with the resistance of many clones not only to β-lactams but also to other antibiotic classes.

Many of the early or so-called" archaic" MRSA clones were derived from the "First MRSA" strain, later designated as sequence type (ST) 250 by MLST technique (multilocus sequence typing). The archaic MRSA clones determined HAIs firstly in Europe until the 1980s. At that time, new and predominant strains of MRSA emerged worldwide, being the cause of the third wave of resistance in *S. aureus*, that continued even in the 21st century [91]. During the 1980s, MRSA spread globally to a great extent in many countries which report MRSA rates about 50% or even higher among *S. aureus* stains isolated in hospitals [92]. Beginning in the 1980s, MRSA spread globally at a high level, thus many countries report MRSA rates of 50% or higher among *S. aureus* clinical isolates

The USA300 CA-MRSA clone was thought to have arisen due to the acquisition of another gene, named the speG gene, which is located on the arginine catabolic mobile element (ACME) and is involved in detoxifying harmful host-derived polyamines [66,86].

CA-MRSA is a threatening for human health. A local CA-MRSA of clinical origin clone, with ST8/SCC*mec*IVl (CA-MRSA/J) has emerged in Japan, and it is known to be associated with a severe evolution of impetigo, from bullous form to a potentially fatal invasive infection. Usually, CA-MRSA clones cause skin and soft tissue infections, and occasionally invasive ones. Some staphylococcal proteins, cell-wall anchored (CWA) and covalently linked to peptidoglycan are considered to be involved in adherence, invasion, and immune escape [93]. This clone CA-MRSA/J also harbor virulence genes; for example, the *spj* gene is located on SCC*mec*IVl, and encodes a CWA protein and also the *S. aureus* pathogenicity island SaPI where are located the genes for staphylococcal superantigens (*tst*, *sec*, and *sell*) [76], transferred by a bacteriophage. CA-MRSA/J is widely spread mainly by bullous impetigo in children (a localized blistering skin disease), and by public transport and occasionally causes invasive infections [94]. This clone was also isolated from meats samples [93].

2.5. Biofilm Formation and Tolerance to Antimicrobials

Bacteria are, by definition, unicellular organisms that are present in all natural environments in this state of the free or planktonic cells, but they are predominant as adherent cells to any available surfaces and developing microcolonies which form by confluence large and multilayer communities named biofilms, compared with bacterial citadels [4,18]. In these communities, also compared with multicellular organisms, the cells are encased in a common matrix and manifest a specific architecture and a modified behavior, being similar to eukaryotic tissues in their capacity to communicate and establish a metabolic cooperation, properties assured by the process of intercellular signaling by QS mechanism [74,95,96].

The biofilm matrix surrounding bacteria is contributing to cells' protection against stress conditions and is one of the causes of their tolerance/behavioral resistance to all types of antimicrobials [95].

The biofilm formation is a multi-step and cyclic process in four stages [74]: (1) Reversible attachment of planktonic cells to the surface (celular/ inert surface); (2) Irreversible adherence by adhesin synthesis; (3) Biofilm maturation, by cell multiplication and matrix synthesis, with a great phenotypical and genotipical heterogeneity of biofilm encased cells; (4) Biofilm detachment and dissemination of single cells or aggegates (Figure 2). For *S. aureus* biofilm, the process is initiated when free, floating or planktonic cocci attach to an available surface, on the so-called conditioning film of host proteins associated with a cellular substratum or with a catheter /medical device which sustains bacterial growth, multiplication and colonization [4,97,98]. *S. aureus* adherence, initially reversible, thereafter irreversible (dependent of adhesin synthesis) to a surface is influenced by hydrophobic and hydrophilic interactions between the bacteria and the surface of any biotic or abiotic surface [99]. The formation of microcolonies is dependent on cell multiplication and synthesis of matrix polymers (EPS), leading gradually to the formation of the biofilm matrix and a mature biofilm [100]. Once the biofilm reaches its mature form, the deep biofim cells, more and more starved, produce different substances, i.e., D-amino acids and EPS-degrading enzymes such as alginate lyase, autolysin, to break and disperse the biofilm as single cells or aggregates [101]. Final dispersal is mediated by the virulence regulator Agr system via secreted enzymes and PSMs [5]. These free cells /aggregates are able to resume the cycle and recolonize a nearby site or to be disseminated at distance, repeating the process and forming a new biofilm in a favorable environment. The biofilm formation is controlled by multiple regulatory systems [5,14,102].

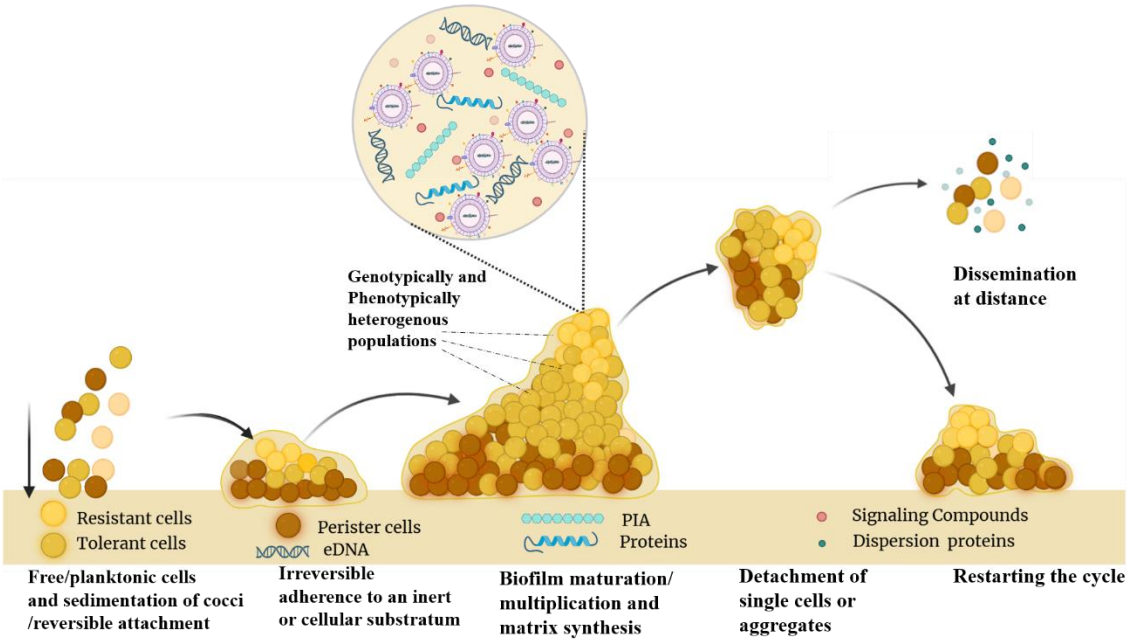

Figure 2. The multi-step and cyclic process of biofilm formation (original image created with www.BioRender.com, accessed on 17 January 2025).

During the biofilm formation, *S. aureus* expresses a large number of adhesins, represented by surface proteins covalently linked to peptioglican layers, known as CWA proteins. Among these molecules, the most prevalent are those designated as MSCRAMMs are the most prevalent [4,72].

Progression from adherent cells, to microcolonies and a biofilm community is dependent of bacterial division and secretion of matrix components, accompanied by a gradually increased physiological heterogeneity of encased cells, influenced by gradients of nutrients and oxygen [72]. Thus, the cells of a mature, multilayer biofilm composed by aerobic strictly or facultative bacteria (such as *S. aureus*) are belonging to four metabolic states: (i) cells with aerobic respiration (at the surface layer of biofilm, oxygenated and nutrient-rich); (ii) cells with fermentative respiration (inside the biofilm, where oxygen and nutrients are less avalilable); (iii) dormant or persistent cells (in anoxic layer, starved cells with slow growth or non-growing cells due to the entrance in metabolic latency); and (iv) dead cells [103]. Dormant or persister bacteria, due to metabolic latency and adenosine triphosphate (ATP) depletion, become less sensitive to antibiotics and all kind of antimicrobials [72,104].

The entrance in the metabolic latency or dormant state is probably the main cause of the tolerance to bactericidal doses of antibiotics and other antimicrobials; the matrix too is acting such as a diffusion barrier for antimicrobials; in the same time, exposure to stress conditions is associated with an increased frequency of mutations (with survival value), with a change in genes' expression by a QS regulated-mechanism (activation/repression). Entering in metabolic latency state the bacterial cells become insensitive to antibiotics which are generally acting by inhibiting different metabolic reactions. All these factors contribute to tolerance/ survival/ persistence of biofilm cells [20].

In *E. coli*, the toxin–antitoxin modules are correlated with persisters' formation, but in Gram-positive bacteria the mechanism is different [105]. The entrance of *S. aureus* cells into the persistent status is correlated with the stationary phase and the reduced level of intracellular ATP, a biomarker of the persister cells. Another biomarker of biofilm cells has been also identified. It is a non-ribosomally synthesized peptide called aureusimine (phevalin), produced in high levels by *S. aureus* biofilm cells [106]. In the same time, entering in this stationary phase the cells become more resistant, with a 100 to 1000-fold of antimicrobial doses, determined by the standard method of the minimum inhibitory concentration (MIC). assay on cells in suspension [104]. It is obvious that such doses are impracticable for medical purpuses, but also in the external environment. It is the reason for the great interest in research of altenative or complementary solutions to antibiotics/ antimicrobials, to efficiently combat the biofilm associated infections (BAIs), but also for an effective disinfection of contaminated surfaces/equipments and others in medical settings and in food industry.

Biofilm matrix. The staphylococcal biofilm matrix has been investigated by a lot of researchers who reported data about its heterogeneity and variability [107]. Similar to other bacterial biofilms, it containing 97% water. The

matrix contains eDNA, derived from lysed bacteria and possibly from host dead neutrophils, and is targeted by DNAses, which contributes to biofilm dispersal [108,109]. The staphylococcal biofilm matrix also contains proteinaceous adhesins, associated with bacteria; this part is formed of amyloid fibers, with a scaffold role and contributing to biofilm development formation and persistent BAIs, and also acting as toxins against bacterial competitors or even host immune cells [96]. The same cytoplasmic proteins have been identified as matrix components and probably have a certain function [110]. Staphylococcal biofilm matrix also contains the specific adhesin—PIA, a major component of the staphylococcal biofilms, especially in certain strains of *S. epidermidis* [66]. Both predominantly proteinaceous or polysaccharide biofilm matrix are susceptible to degradation by proteases and polysaccharide-degrading enzymes [111]. Teichoic acids have also been identified in the biofilm matrix, and being highly charged, they seem to be critical for *S. aureus* colonization of abiotic surfaces [14]. Some authors consider that other cellular components are also present, but they need more studies [5].

Staphylococcal biofilm growth is associated with foreign bodies [112], by also with adherence and BAIs, such as skin infections, abscess, septic endocarditis, osteomyelitis, infection and persistance in cystic fibrosis lung, urinary tract infections [5]. The local conditions for staphylococci to develop an infection are important in all cases, and different from an infection on a cellular substratum which needs synthesis of specific virulence factors: enzymes, toxins and superantigens [24,113], to those needed for adherence and biofilm formation on an inert material/ implant. Firstly, a much lower bacterial cells number is necessary (estimated at 10 000-fold lower) to adhere and colonize an inert surface, by comparison with the minimal number to cause a skin abscess [114]. That is explained by the absence of vascularization at that site, and probably by a lower presence of innate immunity factors [112]. It is proved that agr QS-regulatory system is not essential for initiation of a staphylococcal implant associated infection [115]. Thus, it is considerably important the context of infection when there are analyzed the contrasting results of diferent studies [5].

S. aureus can readily form biofilms even in acidic conditions [116] and their presence amplifies the drug-resistance, even if their counterpart—free, single cells showed susceptibility, and this particularity leads to difficulties for eradication of BAIs at different tissues/organs level [18].

According to NIH (National Institutes of Health—USA) reports, about 80% of human infections are biofilm associated infections [117]. Even since 2000, the CDC (Center for Disease Control—USA) has stated that BAIs represent one of the major challenges for the medical community, which must find solutions to reduce catheter-associated infections and implicitly the duration of hospitalization, mortality from respiratory tract infections, infections that occur in patients receiving long-term medical care [18]. For example, worldwide reports show that Gram-positive bacteria such as *S. aureus*, but also *S. epidermidis* and other coagulase-negative staphylococci, are the most common etiological agents of catheter site infection and peritonitis associated with peritoneal dialysis [118]. There are a lot of reports with experimental data showing the huge tolerance (around 1000-fold and even more), justiying the effors of researchers to find and develop new antibiofilm agents against *S. aureus* and other biofilm formers [4,72,119].

2.6. Prevention, New Antimicrobial and Anti-Biofilm Strategies

Concerning the curently strategies to combat the *S. aureus* AMR detemined by plasmidial or cromosomal resistance genes and the disemmination of present clones and emergence of others, there are two directions of action: decontamination procedures of MRSA carriers, and identification of new antimicrobials or alternative to antibiotics. AMR and biofilm-developing capacity contribute to the success of *S. aureus* as a pathogen in both healthcare settings, as well as in community. It is proved that the expression of virulence factor is coordinated and the biofilm phenotype of clinical isolates is influenced by acquisition of the gene *mecA* (methicillin resistance).

Decolonization of carriers. MRSA colonization is associated with an increased risk of infection and contributes to transmission. Both MRSA colonization on hospital admission as well as acquisition during hospitalization are associated with an approximately tenfold increased risk of subsequent infection [117]. Thus, decolonization of MRSA carriers can contribute to MRSA control, reducing dissemination and infection risk. Generally, decolonization strategies use topical antimicrobials applied into nostrils, that are the main site of colonization [51]. Mupirocin (pseudomonic acid A) which is widely used, acts by inhibitition of isoleucyl tRNA synthetase, preventing protein synthesis. Even mupirocin is the actual best solution for eradication of *S. aureus*, due to the increasing resistance, it is recommended to be used judiciously and to monitor the resistance level, as well reseach for development of new agents [120].

Short-term decolonization. This type of decolonization was applied in the Intensive Care Units (ICUs) setting in three variants and the results of a large cluster-randomized trial (CRT) have been variable [121]. In fact, there were compared three strategies: (1) screening and isolation of MRSA carriers (no decolonization); (2) screening,

isolation and decolonization of MRSA carriers (by standard procedure: mupirocin and chlorhexidine bathing) (targeted decolonization); (3) decolonization of all patients (universal decolonization). The results showed that there are no significant differences in MRSA colonization and infection rates, following the three strategies. But a significant observation was that among the patients of the third group (universal decolonization) the bloodstream infections with any pathogen were significantly lower. The authors considered that this result is mostly due to universal chlorhexidine bathing rather than mupirocin and concluded that universal decolonization was the best approach (without the need for screening). But, due to the risk of drug resistance emergence by an excessive use of topical antibiotics, their use should be coupled with resistance monitoring [14,120].

Anti-biofilm strategies. The high incicidence of BAIs and their tolerance or recalcitrance to multiple antibiotics have boosted research towards discovering alternative strategies to antibiotherapy. There are already now a lot of inhibitory substances or antagonist species, with antiinfectious therapeutical potential.

In the latest articles a lot of researchers report data on various anti-biofilm substances discovered or tested to date in vitro, in vivo on experimental models, and some of them even in humans. We recently revised such alternative or complementary strategies to antibiotics [18] that include plant active compounds, with antimicrobial and anti-biofilm effects (extracted from herbs, leaves, tree bark, fruits, seeds) [122,123] and with different chemical structure—essential oils (EOs) [124,125], phenolic compounds, propolis—a natural and complex bee product [126], but also bacteriophages, bacteriocins/ lantibiotics, enzymes [127], AMPs [75], nanoparticles [18], biological methods based on the interspecific antagonism (i.e., competition/probiotics [128] or predator organisms [129], physical modern methods based on light or ultrasounds for biofilm removal, new synthetic chemical compounds. Currently a good, efficient solution is considered the combined therapies, with antibiotics, other antimicrobials, i.e., nanoparticles [97], EOs [130], chelating agents, and immunological methods (vaccines, monoclonal antibodies) or QS inhibitors (QSIs) [18].

About BAIs produced by oppotunistic pathogens, one current strategy is represented by the anti-pathogenic or anti-virulence strategy by QSIs and *quorum* quenching enzymes (QQ) [131], which target the virulence genes' expression or their products. So, this strategy is in turn divided into two types: one is targeting *S. aureus* toxins and the second one is targeting the coding genes expression [66]. In order to interfere with pathways involved in virulence factors' production, it is essential to study and understand the signaling pathways, the mechanisms of pathogenesis, and implicitly to find new ways to combat pathogens intelligently, without inducing or amplifying antimicrobial resistance.

Since the STX is considered as an important virulence factor of *S. aureus*, among the anti-virulence strategies, the STX biosynthesis pathway is one of the targets against infections. There are many studies focused on the inhibition of STX production and it was proved that by interference with the biosynthesis way of STX the virulence of pigmented *S. aureus* isolates declined [132].

Many previous studies reported a lot of substances identified as potential anti-virulence candidates. For instance, variants of peptidic autoinducers (AIPs) specific for Gram positive bacteria have demonstrated the capacity to inhibit the virulence of MRSA clones. For example, a thiolactone structure, the cyclic AIP mimetics have been proved to reduce in vitro the accessory gene regulator histidine kinase (AgrC) function [133]. A family of small-molecule compounds, phenolic components of traditional Chinese medicinal herbs (β-cyclodextrin), have been proved to inhibit α-hemolysin (Hla) in vitro production, and thus reducing *S. aureus* colonization. In addition, some of them could also significantly supress the process of gene transcription for toxins *sea*, *seb* and *tsst-1* [88,134].

Starting from in vitro observations about the anti-virulence effects of probiotic supernatants, subinhibitory concentrations of phenyl-lactic acid (PLA), the main microbicidal metabolite of lactic acid bacteria, were able to attenuate the virulence and pathogenicity of *S. aureus* clinical strains, an anti-pathogenic effect demonstrated by an in vivo experimental infection [128]. The knowledge about the communication systems and regulation of bacterial virulence genes offered a new antiinfectious drug target, without interfering with bacteria growth. Thus, the inhibition of QS genes expression may represent a new strategy for attenuation of virulence and pathogeneicity, including of *S. aureus* clinical strains. Between the QSIs of different origins, there are also those of microbial origin, synthesized by probiotic bacteria, representing thus an interesting, new anti-microbial strategy for the prevention and therapy of staphylococcal infections [135].

In this context, the research currently focuses on the discovery of new antibiotics, but also of innovative drugs/therapies, alternative strategies for an efficient combat of MDR pathogens and biofilm formers.

2.7. Interspecies Relationships—Examples and Advantages

In all ecosystems, living organisms, including microorganisms, compete for resources, which are important for their survival [136]. The interspecific antagonism is well known as phenomenon, with some examples, but less exploited; in the last time the researchers are attracted by this possibility, to fight efficiently against pathogens. Here, we present several examples of *S. aureus* antagonists.

The organisms of the nasal microbiota are in competition with each other in several ways. For example, they compete for adhesion sites and nutrients: there are low amounts of nutrients in the human nose. *S. aureus* can survive in environments with lower levels of nutrients than coagulase-negative staphylococci, possibly owing to differences in metabolism, and hence is better adapted to the human nose. However, no difference in nutrient levels has been observed between carriers and non-carriers. Microbiota species compete also by antibiosis, producing antimicrobial molecules that inhibit their competitors. For instance, *S. lugdunensis* produces an antimicrobial compound called lugdunin able to inhibit and destroy *S. aureus* cells (including MRSA) in vitro and in a mouse model, possibly by leading to rapid breakdown of bacterial energy resources [51,137]. In humans, nasal colonization with *S. lugdunensis* has been associated with a six-fold lower risk of colonization with *S. aureus*. These findings are certainly interesting, but explain only a minority of carriage patterns, as *S. lugdunensis* colonization has been reported in only 9–26% of the general population [14].

S. epidermidis molecules reduced biofilm formation of *S. aureus*, including agr-positive and agr-defective strains and also disintegrated established biofilms and reduced the necessary dose of antibiotics to eliminate them. Therefore, such molecules able to counteract the biofilm formation may be promising alternatives to control *S. aureus* infections [138,139]. There are studies reporting about the potential of *Bellovibrio bacteriovorus* (a predator bacterial species) to be used as an alternative solution, being a real "living-antibiotic". There are already data about the significant potential of *B. bacteriovorus* to kill MDR bacteria often implicated in the etiology of the nosocomial infections, such as *Staphylococcus aureus*, the other members of ESKAPE group and *Escherichia coli* [129].

The current antibiotics crisis imposes the search for new therapeutic strategies, without the disadvantage to induce drug resistance. In recent years, the anti-pathogenic strategies, targeting virulence factors or their coding genes expression have been accepted as possessing a great potential, representing an alternative to antibiotics [88].

3. Conclusions and Perspectives

During the latest several decades, the AMR continously evolved due to the long-term and misuse (sometimes unnecessary use) of antibiotics, currently driving to the frequent unsuccessful treatments with available antibiotics.

A biofilm it is not only a dense community; it is also a social arrangement, with a real "labor division", with a great metabolic heterogeneity of the stratified component cells, sensing the signals from environment, communicationg permanently each other by QS mechanism and giving an adequate response. The main result of biofilm formation is their tolerance or recalcitrance to all stress factors, including all kind of microbials. So, using QSIs or QQs for efficiently fight against biofilm associated infections is a non-conventional approach, an intelligent strategy, based on interruption of the communication systems and all density dependent gene expression and functions. It is obvious that antibiotics act by inhibiting bacterial growth or cellular viability and, as selective pressure factors, induce resistance. The anti-pathogenic strategies targeting bacterial virulence genes expresion might be less possible to develop drug resistance.

There are already some new antimicrobials and anti-biofilm drugs with a great therapeutical potential, combined and innovative therapies, in different research steps, most of them needing to be validated by clinical studies. There is necessary more research, in many promising directions, including the interspecific antagonism that is well known, less exploited, but with a great potential in the future.

Author Contributions

Conceptualization and supervision: V.L. Writing–original draft preparation: V.L. and V.-A.B. Preparing the figure artwork: V.-A.B. Editing: R.M.A. and L.-M.D. All authors have read and agreed to the published version of the manuscript.

Funding

This research received no external funding.

Institutional Review Board Statement

Not applicable.

Informed Consent Statement

Not applicable.

Data Availability Statement

The data underlying this article will be shared on reasonable request from the corresponding author.

Conflicts of Interest

The authors declare no conflict of interest.

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