Omics Approaches for the Study of Adaptive Immunity to *Staphylococcus aureus* and the Selection of Vaccine Candidates

Silva Holtfreter 1,*, Julia Kolata 2, Sebastian Stentzel 1, Stephanie Bauerfeind 1, Frank Schmidt 3, Nandakumar Sundaramoorthy 3 and Barbara M. Bröker 1

1 Department of Immunology, University Medicine Greifswald, Greifswald 17475, Germany; sebastian.stentzel@uni-greifswald.de (S.S.); stephaniebauerfeind@web.de (S.B.); broeker@uni-greifswald.de (B.M.B.)

2 Department of Medical Microbiology, University Medical Center Utrecht, Utrecht 3584, The Netherlands; J.B.Kolata@umcutrecht.nl

3 ZIK-FunGene Junior Research Group Applied Proteomics, Department of Functional Genomics, Interfaculty Institute for Genetics and Functional Genomics, EMA-University of Greifswald, Greifswald 17475, Germany; frank.schmidt@uni-Greifswald.de (F.S.); sundaramon@uni-greifswald.de (N.S.)

* Correspondence: silva.holtfreter@uni-greifswald.de; Tel.: +49-3834-865518

Abstract: *Staphylococcus aureus* is a dangerous pathogen both in hospitals and in the community. Due to the crisis of antibiotic resistance, there is an urgent need for new strategies to combat *S. aureus* infections, such as vaccination. Increasing our knowledge about the mechanisms of protection will be key for the successful prevention or treatment of *S. aureus* invasion. Omics technologies generate a comprehensive picture of the physiological and pathophysiological processes within cells, tissues, organs, organisms and even populations. This review provides an overview of the contribution of genomics, transcriptomics, proteomics, metabolomics and immunoproteomics to the current understanding of *S. aureus*-host interaction, with a focus on the adaptive immune response to the microorganism. While antibody responses during colonization and infection have been analyzed in detail using immunoproteomics, the full potential of omics technologies has not been tapped yet in terms of T-cells. Omics technologies promise to speed up vaccine development by enabling reverse vaccinology approaches. In consequence, omics technologies are powerful tools for deepening our understanding of the “superbug” *S. aureus* and for improving its control.

Keywords: *Staphylococcus aureus*; vaccine; immune response; adaptive immunity; genomics; proteomics; transcriptomics; immunoproteomics

1. Introduction

*Staphylococcus (S.) aureus* is a Janus-faced microorganism: On the one hand, about 20% of human adult population are persistently colonized with this bacterium, usually without clinical symptoms [1]. On the other hand, *S. aureus* is notorious for causing a broad range of infections and for rapidly evolving resistances [1,2]. These bacteria are the second most common cause of hospital-acquired infections in general and the leading cause of skin and soft tissue infections, nosocomial pneumonia, wound infections, and Gram-positive sepsis in particular [3–5].

The high incidence of *S. aureus* infections is due to the expression of a broad variety of bacterial virulence and immune evasion factors and to the rapidly evolving resistance to antibiotics [2,4,6–8]. Methicillin-resistant *S. aureus* (MRSA) are spreading in hospitals as well as in the community [4,9], and *S. aureus* strains resistant to last reserve antibiotics are reported worldwide. Worryingly, no new classes
of antibiotics have been introduced to the market by the pharmaceutical industry over the last three decades. Hence, we may be facing a future where \textit{S. aureus} cannot be treated efficiently anymore [10]. These alarming perspectives are calling for additional preventive and therapeutic strategies, such as vaccination and novel anti-microbial therapies. To date, however, all \textit{S. aureus} vaccine trials have failed [11–13].

Omics technologies provide panoramic views of the molecular determinants of life and their interactions enabling an unbiased approach to physiological and pathological processes. The methods (with the exception of metabolomics) are grounded in genomics, which was sparked by the sequencing of the complete genome of the bacterium \textit{Haemophilus influenzae} in the year 1995 [14]. The successful deciphering of the human genome in the year 2001 marks another milestone [15,16]. Omics studies generate detailed and comprehensive insights on the information content of DNA (genomics), its temporal transcription into RNA (transcriptomics), and its translation into proteins (proteomics) and metabolites (metabolomics). Furthermore, immunoproteomics provides an overview of immunogenic peptides or proteins. The resulting broad perspective can complement targeted strategies that aim at elucidating the functions of single factors in cause and effect chains.

Previous attempts to translate promising pre-clinical results into a successful vaccine for patients have given disappointing results. Omics technologies are a powerful tool to help overcome the hurdles by increasing our knowledge about the mechanisms of protection. This review starts with a brief outline of current challenges in studying adaptive immunity to \textit{S. aureus}. Following this, it provides an overview of the contributions genomics, transcriptomics, proteomics, metabolomics and immunoproteomics to our current understanding of:

- the \textit{in vivo} behavior of \textit{S. aureus};
- the antibody and T-cell response against \textit{S. aureus}; and
- vaccination development.

### 2. Challenges in Deciphering the Adaptive Immune Response to \textit{S. aureus}

In order to be able to prevent or treat \textit{S. aureus} infections in the future, we need to learn more about the \textit{in vivo} behavior of these bacteria and the immunological mechanisms of protection. However, such studies are impeded by various factors, such as:

- the diversity and complexity of \textit{S. aureus} host interactions;
- the impressive genetic variability of the species \textit{S. aureus};
- a deficit in infection-relevant \textit{in vitro} and \textit{in vivo} models; and
- the high variability of the anti-staphylococcal immune responses.

Firstly, \textit{S. aureus} host interactions are multifaceted. On the one hand, \textit{S. aureus} is a frequent colonizer of the human skin and mucosa: Around 20\% of adults are persistent carriers of this microorganism; the others are intermittently colonized [17]. However, intensive exposure to \textit{S. aureus} in carriers is a risk factor for infection upon hospitalization [18,19], which in most cases is caused by the colonizing \textit{S. aureus} strain [20,21]. On the other hand, \textit{S. aureus} is a prominent pathogen [1,2], causing skin and soft tissue infections, wound infections, osteomyelitis, pneumonia, and sepsis [3–5]. For decades, \textit{S. aureus} has been considered a classical extracellular pathogen. However, it has become evident that \textit{S. aureus} can survive in different types of non-professional phagocytes, such as epithelial and endothelial cells [22,23]. This ability to invade host cells, to escape from the lysosomal degradation machinery and to persist within the intracellular location most likely contributes to long-term persistence and recurrent infections [22]. Both the bacterial behavior and the mechanisms of protection probably depend critically on the type of infection.

Secondly, \textit{S. aureus} research is challenged by the genetic variability of the species. Two strains can differ in up to 25\% of their gene content [24,25]. Hence, data obtained with a single strain cannot be easily generalized. This species variability fuels a continuous discussion as to whether conserved
or variable (but disease-specific) antigens should serve as vaccine targets. There is consensus that a multivalent vaccine that reflects the genetic diversity of the *S. aureus* species will be superior to a monovalent vaccine [26–28].

Thirdly, the characterization of infection-related properties of *S. aureus* is currently hindered by a lack of data generated from *in vitro* and *in vivo* models that closely mimic infection in humans. Bacterial behavior has been studied extensively in very simplified systems, e.g., by adding stressors to broth cultures. There is an increasing awareness that *in vivo* virulence regulation can differ substantially from that seen *in vitro* [29–32]. Therefore, the focus is now shifting to more complex model systems, such as cell cultures, animal models, and human samples. This shift is promoted by advances in omics techniques. Additionally, recent studies have highlighted the host-specificity of numerous virulence factors and evasion molecules, which needs to be considered when choosing the appropriate animal model [33–37].

Finally, understanding adaptive immunity to *S. aureus* is challenged by the variability of the human host and the induced adaptive immune responses. Adaptive immunity in each individual is shaped by the type of *S. aureus* strain and the micro-environmental context in which the host encounters the bacteria (reviewed in [38]). Therefore, it is of no surprise that the antibody patterns within the human population are highly variable, regarding both specificities and titers [39–41]. Compared to antibodies, relatively little is known about specific T-cell responses against *S. aureus* in humans [38].

3. Omics Technologies in *S. aureus* Research

The remarkable success of *S. aureus* as a colonizer and pathogen depends on its ability to adapt to different environments, e.g., abiotic surfaces such as prosthetic implants and catheters [42,43], the mucosa of the nose, the gastrointestinal and reproductive tract, as well as—upon invasion—virtually every human tissue [1]. Survival in such a wide variety of environmental niches requires adaptation responses, which can affect all regulatory levels from gene to transcript, protein and metabolite. Omics technologies provide powerful tools to study *S. aureus* pathophysiology and host interaction in its entirety and complexity in different experimental set-ups such as cell cultures, animal models, and human samples (Table 1).

3.1. Genomics

Understanding of adaptive immunity to *S. aureus* requires knowledge about the spatial and temporal prevalence of virulence genes in the *S. aureus* population as well as about virulence gene sequence variability.

This area of research has benefitted greatly from whole genome sequencing and DNA microarrays, which have revealed an impressive genetic diversity of the species *S. aureus*. Around 25% of the 2600 genes of *S. aureus* are variable, encompassing the so-called core variable and the variable subgenomes [25]. The core-variable genome accounts for 10% of the genome and comprises, for example, lineage-specific variants of surface adhesions and regulators. Another 15% of the staphylococcal genes belong to the variable genome. These genes are located on mobile genetic elements, such as phages, plasmids and pathogenicity islands [24]. The encoded variable subproteomes are enriched in virulence factors and are therefore highly relevant for host pathogen interaction [24,25]. Whole genome sequencing has been very successfully applied to study *S. aureus* evolution, even in a single individual during colonization or infection, as well as bacterial transmission during outbreaks [44–51]. By comparing virulence gene contents of *S. aureus* isolates from different clinical cohorts and the general population, comparative genomics can indicate targets for vaccination but also provide insights into the molecular basis of pathogenicity [52].
Table 1. Overview on omics technologies and their potential applications for deciphering the behavior of *S. aureus* and the host response under infection-relevant conditions.

| Omics technologies | Genomics | Transcriptomics | Proteomics | Immunomics | Metabolomics |
|--------------------|----------|-----------------|------------|------------|--------------|
|                     | 2nd generation sequencing methods | Microarray | 2D-gel-based proteomics in combination with mass spectrometry | 2D-immunoblot | Mass spectrometry |
| Omics technologies | 3rd generation sequencing methods | RNAseq | Gel-free proteomics | Automated 1D immunoblot | Nuclear magnetic resonance (NMR) spectroscopy |
| DNA microarray | | | Protein microarrays | Suspension arrays | |
| Approaches for deciphering the behavior of *S. aureus* | Sequencing of clinical *S. aureus* isolates | Transcriptomics of clinical *S. aureus* isolates | Proteomics of clinical *S. aureus* isolates | | |
| | Elucidation of the pangenome of the species *S. Aureus* | Genome-wide expression profiles of *S. aureus* under infection-relevant conditions | Elucidation of the panproteome and subproteomes under infection-relevant conditions | | |
| | Detection of gene polymorphisms (SNPs, CNVs) | In vivo transcriptomics | In vivo proteomics | | |
| | Genotyping | Single cell transcriptomics | Metaproteomics of the microbiome of the host | | |
| | Population sequencing (e.g., the entire *S. aureus* population in the host organism). | | | | |
| | T-cell and B-cell epitope prediction | | | | |
| Approaches for deciphering the host response | Detection of gene polymorphisms (SNPs, CNVs) | Genome-wide expression profiles of host cells, e.g., immune cells | Proteomics of host cells, e.g., immune cells | | |
| | Genome-wide association studies | | | | |
| | Analysis of the *S. aureus*-specific T-cell and B-cell repertoires | | | | |
| | | | Variable and core components of the immunoproteome | | |
| | | | Monitor antibody profiles upon colonization, infection or vaccination | | |
| | | | Identification of protective antibody specificities | | |
| | | | Identification of determinants of antigenicity or the strength of the immune response | | |
| | | | Metabolomics of host cells, e.g., immune cells | | |
| | | | Metabolomics of body fluids | | |
Based on genome information, DNA microarrays have been developed. They have provided valuable insights into the genetic variability of *S. aureus* in the general population, in hospital settings and defined patients groups [53–55]. Due to their rapid and simple handling and straightforward data analyses, custom or commercial DNA microarrays are still widely used to obtain an overview of the repertoire of antibiotic resistance and virulence genes [56–63]. However, in comparison to next-generation sequencing, DNA microarrays are of lower resolution, and they do not allow the identification of unknown virulence factors.

From a host perspective, genome-wide association studies uncover host gene polymorphisms associated with bacterial colonization or certain clinical pictures. The state-of-the-art host genetic susceptibility to *S. aureus* carriage and infections has recently been reviewed by Shukla and colleagues [64]. Most investigations into host genetic determinants of *S. aureus* nasal carriage used a candidate gene approach [65–68]. A few studies tried to identify host polymorphisms associated with *S. aureus* disease on a genome-wide level but failed [69–71]. Future investigations using larger sample numbers and narrowed phenotypes as well as building on advances in both genotyping and analytical methodologies will offer the chance of identifying new genetic variants important for *S. aureus* colonization and infections.

Focusing on the immune defense, several elegant methods are available to decipher the T-cell receptor and B-cell receptor repertoires [72–75]. They are based on next-generation sequencing and have already been used for investigations of the T-cell receptor repertoire in cancer, autoimmune diseases and viral infections. In terms of TCR sequencing upon bacterial stimulation, Li *et al.* have profiled the TCR repertoire in patients with pleural tuberculosis [76]. Diluvio *et al.* utilized this method to confirm that, in patients suffering from psoriasis vulgaris, certain TCR beta-chain variable region (TCRBV) genes are clonally expanded in the skin lesions. Interestingly, if these patients additionally developed streptococcal angina, tonsillar T-cells with identical TCRBV genes as in the psoriatic skin lesion were clonally expanded [77]. For addressing the spectrum of *S. aureus*-specific T-cells, T-cell receptor sequencing has not been employed so far.

Concerning rational vaccine design, pan-genomics and comparative genomics enable a novel approach to vaccine development termed reverse vaccinology, an unbiased discovery process for candidate vaccine antigens (see Section 4).

### 3.2. Transcriptomics

High-throughput transcriptomics can reveal changes in gene expression profiles of both the pathogen and the host cells under infection-related conditions as well as during infection. This information is relevant for studying adaptive immune responses to *S. aureus* and also for vaccine development, because only those antigens that are actually expressed *in vivo* are vaccine candidates.

Transcriptomes of bacteria and host cells can be profiled using microarrays as well as next-generation RNA sequencing technologies (RNAseq) (Table 1). Current microarrays contain millions of probes on a single chip, enabling the simultaneous measurement of gene expression of a multitude of genes. Microarray analyses for studying *S. aureus* host interaction *in vitro* require sufficient starting material, *i.e.*, around 5 µg RNA, which corresponds to 5 × 10^5* host cells and 1 × 10^8* bacterial cells. RNAseq employs DNA deep sequencing technology to sequence all RNA transcripts within a sample. The technique is rapidly replacing traditional expression microarrays as the method of choice for determining global gene expression profiles in bacteria and host [78–81]. It requires less material than RNA microarray approaches, and provides the possibility to determine absolute transcript levels of sequenced but also of non-sequenced organisms. Moreover, this method is able to identify novel transcripts and RNA isoforms. For validating data from microarray and RNAseq experiments, qRT-PCR is the method of choice.

In the past, great efforts have been made toward the mapping of transcriptomes of *S. aureus* laboratory strains and clinical isolates under environmental stressors in cell culture, such as aerobic versus anaerobic growth, antibiotics, oxygen radicals, nitric oxide or iron limitation [82–87]. However, recent data has shown that the *S. aureus* transcriptional profile during growth in broth culture may correlate poorly with gene expression in mammalian colonization and infection models [29–32].
Therefore, the focus is now shifting to more complex model systems that mimic the S. aureus host interaction more closely. For example, transcriptome analyses were performed on S. aureus cells grown in body fluids, i.e., serum, blood, pulmonary surfactant, artificial nasal secretions or sputum medium [88–92]. Moreover, transcriptomics provides insight into transcriptional adaptation to different staphylococcal lifestyles, such as intracellular survival in professional or nonprofessional phagocytes, small colony variants (SCVs) and biofilm formation [93–95].

So far, most studies have analyzed transcription profiles of either bacterial cells [95,96] or host cells [97–104], while studies combining both biological systems are still rare. In a pilot study, Depke studied host gene expression in a kidney abscess model using transcriptomics and simultaneously monitored the expression of selected S. aureus genes by RT-qPCR [105]. Regarding the adaptive immune response to S. aureus, especially that of T lymphocytes, transcriptomics has been limited to the investigation of certain target genes by real-time-PCR [106–110]. In our group, T-cells of healthy individuals were isolated from PBMCs, stimulated with recombinant S. aureus antigens, and the transcription profiles of the resulting T-cell lines were analyzed using array technology. In line with the data from mouse experiments, these cells predominantly but not solely responded in a Th1/Th17 manner (Steinke, personal communication).

The grail, however, is the study of gene regulation and gene expression in real life, namely, infected or colonized animals or patient material. Characterizing host responses during resolution of S. aureus infection will allow the definition of immune correlates of protection. The amount of RNA required and the vast excess of host RNA over bacterial RNA in most infected tissues are limiting the application transcription profiling to infections with low bacterial densities or asymptomatic colonization. Gene expression analysis during persistent colonization has been studied using quantitative RT-PCR as well as recently via RNASeq [29,81,111]. The data of the direct ex vivo RNASeq approach corroborate results obtained with an artificial nasal medium and show that the nasal micro-environment imposes iron and nutrient limitation stress upon the bacteria [81,112]. Initial studies addressing the S. aureus transcriptome during infection [32,113–115] suggest that each infection type, e.g., pneumonia, endocarditis or abscesses, has its specific signature, due to the presence of organ-specific environmental triggers [86,116].

### 3.3. Proteomics

Proteomics approaches include 2D-gel-based proteomics, shotgun-proteomics, as well as protein microarrays (Table 1). 2D-gel-based proteomics was developed in the 1990s and combines protein separation (2D-PAGE), enzymatic protein digestion, detection of the resulting peptides by MALDI-MS and the bioinformatic analysis of the resulting peptide mass fingerprints (PMF). Moreover, fluorescent dyes enable sensitive in-gel detection of proteins and their quantification over a wide dynamic range [117]. Though the high resolution of protein species, i.e., protein variants encoded by a single gene, is of advantage, this method is limited due to the high manual effort, the required sample size and the selective analytical window of the targeted proteins. 2D-gel-based proteomics has been instrumental in the analysis of the S. aureus proteome [118].

The rapid development of MS techniques with high mass accuracy in combination with novel gel-free sample preparation methods enabled gel-free proteomics, which is superior in terms of comprehensiveness of the acquired data, versatility of the accessible samples, sensitivity, resolution and the required protein amount (ng vs. µg quantities) [119,120]. Moreover, quantitative proteomics workflows have been implemented using isotopic labeling or even label-free analyses [121].

When applying proteomics to the analysis of pathogen and host, it is—in contrast to the analytics of nucleic acids that can cover the entire genome—usually impossible to catch the entire proteome in one single protein preparation. Cytosolic, membrane and membrane-associated, cell surface-exposed, and secreted proteins need to be analyzed separately [120,122,123]. Using sophisticated methods for the sub-fractionation of the staphylococcal subproteomes, Becher et al. were able to provide a very comprehensive protein inventory of living bacteria, including quantitative data for almost 1700 S. aureus proteins, corresponding to 80% coverage of all expressed proteins [118]. Highlighting the technical advances in MS, nowadays even unfractionated protein samples may reach an identification
rate of roughly 90% by using high sensitivity mass spectrometry combined with sophisticated peptide enrichment techniques, as shown by Depke et al. [124].

Over the last 15 years, one focus of proteomics application to *S. aureus* research has been on understanding the functional adaptation to stress and starvation as they are encountered by the bacteria during infection and/or antibiotic treatment [120,125–127]. Engelmann and colleagues defined proteome signatures of different stressors, including fermentation, nitrate respiration, diamide stress, H$_2$O$_2$, and nitric oxide [125,128–130]. These signature libraries form a useful toolbox for deciphering the physiological state of bacteria grown under infection-related conditions [120,131,132]. Moreover, they can aid in finding key enzymes and therefore potential key targets for novel antimicrobial therapies [120]. The proteome signature data are publicly available in the database Aureolib [132].

Cell surface-exposed secreted bacterial proteins are subproteomes that are centrally involved in *S. aureus* host interaction. They are enriched for virulence factors, immune evasins and adhesins [123], which are predominantly targeted by the humoral immune response and thus represent candidates for antibody-based vaccines [133]. By dissecting the exoproteomes of 25 clinical *S. aureus* isolates, Ziebandt et al. discovered that their composition was extremely variable; only eight proteins were shared by all isolates [134]. This variability was only partially explained by genome plasticity but mainly resulted from a high degree of expression heterogeneity. These data highlight the importance of combining different omics approaches to obtain a complete picture of the bacterial behavior [134].

An important issue to be addressed in future studies will be the identification disease-specific (exo)proteome signatures as they have been reported in a recent pilot study [135].

In vivo proteomics studies analyzing the functional state of *S. aureus* during colonization or infection are still rare, because they are hampered by the minute amounts of sample that can be obtained in vivo, as well as by the interference by abundant host materials like proteins and/or nucleic acids [120,136]. In order to enrich bacteria from in vitro infection experiments, approaches to separate bacteria from host cells include centrifugation, immune-magnetic separation and fluorescence-activated cell sorting (FACS) [137–140]. The isolation of intracellular compartments such as phagosomes, which contain the bacteria and their secretion products, promises the identification of bacterial proteins that are released by the bacteria during invasion and persistence inside host cells [140–143].

A pilot proteomics study on murine *S. aureus* pneumonia demonstrates that proteomics are feasible for ex vivo samples, such as bronchoalveolar lavages [145].

Focusing on the host immune defense, proteomics, especially when combined with next-generation sequencing [146], opens new avenues for the elucidation of the antibody- and T-cell repertoires [147,148]. This technology has not yet been applied to comprehensively map the T response to *S. aureus*. On a smaller scale, T-cell function is usually addressed by determining the generation of cyto- and chemokines as well as the expression of cell surface markers using multiplex assays. The human cytokinome, comprising all known cytokines and chemokines, has more than 240 members [149]. It is currently not possible to record the whole cytokinome at once due to the large dynamic concentration range of different cytokines. However, advanced bead-based methods are available to simultaneously measure approximately 50 cytokines/chemokines from a single sample [150]. When addressing the antigen-specific cytokine response of T-cells to *S. aureus*, such bead-based multiplex assays are currently the method of choice [151,152].

Cell surface marker expression can be addressed by advanced flow cytometric approaches as well as mass cytometry (e.g., CyTOF technology), enabling the simultaneous assessment of up to 40 parameters. These multiplex approaches allow the analysis of T-cell differentiation upon antigen-specific activation. State-of-the-art mass spectrometry applied to extracts enriched in cell
surface proteins could in the future help to resolve the modulation of the T-cell surfacome in *S. aureus* infection in its full complexity.

### 3.4. Immunoproteomics

Immunoproteomics is a sub-discipline of immunomics, which aims at studying the function and regulation of the immune system in its entirety using omics approaches [147,148,153–155]. Immunoproteomics builds on proteomics for the comprehensive analysis of the adaptive immune response. Using gel-, array-, and mass spectrometry-based techniques, immunoproteomics has the goal of identifying and measuring antigenic peptides or proteins as well as the adaptive immune response directed against them [156,157].

For unbiased anti-staphylococcal antibody profiling, 2D-immunoblotting (2D-IB) in combination with MS has been employed and the immunogenic antigens of *S. aureus* have been mapped (Figure 1). While being labor-intensive, it enables a personalized approach by simultaneously providing information about the virulence factor repertoire of a clinical *S. aureus* isolate (proteome) and the specific antibody response of the affected patient or carrier (immunoproteome) [39,40]. In view of the pronounced heterogeneity of the species *S. aureus* a personalized strategy reduces the experimental noise, and it has revealed rules governing the antibody response to *S. aureus* [38,157,158].

![Workflow of immunoproteomics approaches](image.png)

**Figure 1. Workflow of immunoproteomics approaches.** Schematic representation of three commonly used immunoproteomics-based approaches for the identification and quantification of anti-staphylococcal antibodies. (A) 2D-immunoblots. *S. aureus* proteins are separated based on their pI, followed by gel-based resolution according to their molecular weight. Afterwards, proteins are transferred to a membrane by Western blotting and immobilized. Anti-*S. aureus* antibodies from patient sera specifically bind to their respective *S. aureus* antigen and are visualized by labeled secondary antibodies. Since the bacterial antigens are denatured during resolution on 2D gels, predominantly non-conformational epitopes are detected with this approach; (B) Protein Array. A panel of recombinant or purified *S. aureus* antigens is spotted on a solid surface in an ordered manner. Afterwards, anti-*S. aureus* antibodies in patient sera are detected using labeled secondary antibodies. Proteins can be applied in their native form, allowing the detection of conformational epitopes; (C) Suspension array. Up to 500 discrete assays are performed simultaneously on the surface of distinct color-coded beads known as microspheres. Using multiple lasers or LEDs and high-speed digital-signal processors, an analyzer reads multiplex assay results by reporting the reactions occurring on each individual microsphere. For *S. aureus* immunoproteomics, panels of recombinant or purified *S. aureus* antigens have been coupled to distinct microspheres, and anti-*S. aureus* antibodies can be quantified over a large linear range after incubation with patient serum and labeled secondary antibodies. Additionally, in this case, antigens with conformational epitopes can be detected, if proteins are coupled in their native conformation. Images were adapted from Tjalsma et al. [159].
Immunocapture MS has a similar scope [159]. This technique is based on immobilization of patient antibodies, which are directly used to isolate antigenic proteins from a complex mixture of proteins. The captured antigens are subsequently profiled by MS. This method allows the detection of conformational epitopes, because, in contrast to 2D-IB, non-denatured protein mixtures can be exposed to the immobilized antibodies.

Finally, E. coli surface display libraries have been established for identifying S. aureus antigens that are recognized by antibodies [160]. Here, either uniformly small (linear epitopes) or uniformly medium-sized (potential conformational epitopes) peptides encoded by the bacterial genome are displayed on the surface of E. coli via fusion to outer membrane proteins. The resulting E. coli libraries can be probed with patient sera.

Building on prior knowledge about the antigenic composition of S. aureus, immunogenic S. aureus proteins can be recombinantly expressed for (1) validation of their role as prominent antibody targets and (2) integration into multiplex assays permitting high throughput quantification of specific antibodies (Table 1) [41,133,161,162]. Most multiplex assays used in S. aureus research are based on suspension array technology (e.g., Luminex®) that allows simultaneous quantification of antibody binding to up to 500 bacterial proteins over a large dynamic range (10^5). Alternatively, S. aureus proteins or peptides can be spotted onto protein arrays (Figure 1) [159,163].

3.4.1. Antibody Profiles in Healthy Individuals

Healthy individuals, be they S. aureus carriers or noncarriers, harbor antibodies against a broad spectrum of S. aureus antigens (as reviewed in [39]). As expected, antigens accessible at the cell surface or released by the bacteria are immunodominant over intracellular proteins. The antibody spectrum within the human population is highly variable, regarding both specificities and titers [39–41], which reflects the history of encounters with S. aureus. For example, multiplexed bead-based assays demonstrated that healthy individuals, be they carriers or non-carriers, mount a highly variable antibody response against S. aureus surface and secreted proteins with titers differing by a factor of 1:10 to 1:1000, depending on the tested staphylococcal antigen [41,164] (and Nandakumar Sundaramoorthy, personal communication).

How are these serum antibodies induced? Studies suggest that mere epithelial colonization is not sufficient to trigger a serum IgG response in S. aureus carriers. For example, nasal colonization in infants frequently precedes sero-conversion, both the generation of IgM and IgG, for many months [165]. Moreover, experimental nasal colonization in humans does not elicit a robust serum IgG response [40]. This suggests that the specific immune memory of S. aureus observed in most adults is probably elicited by minor invasive episodes.

The high level of antibodies in noncarriers shows that these persons are also frequently exposed to S. aureus. In fact, when measuring the antibody response to conserved S. aureus antigens, the differences between carriers and non-carriers are small in comparison to the very large variations within the two groups [41,165,166]. However, while carriers will experience repeated episodes of infection with their colonizing strain, resulting in a strong strain-specific immune response [167], non-carriers might contact a wider range of different S. aureus isolates. In line with this, healthy carriers have a robust antibody response to S. aureus, which is even slightly stronger than that in non-carriers [39,41,164,165]. It is obvious that antibodies do not eliminate S. aureus from the body surfaces; there is no sterile immunity to S. aureus. Nor do these antibodies reliably protect from infection [18,19] or from colonization with a different S. aureus strain [168].

3.4.2. Antibody Profiles during Infection

S. aureus-specific antibody patterns in the general population are highly variable. Thus, persons infected by S. aureus will likely be at very different immunological “starting positions,” which might influence the outcome. In line with this, recent data suggest that intensive exposure protects from severe S. aureus disease and death. While carriers acquire an S. aureus bacteremia more frequently
than non-carriers, mostly from their endogenous strain, they have a significantly better chance of survival [18,19]. To explain this, we have proposed that long-term exposure to *S. aureus* in carriers primes the adaptive immune system, likely via repeated subclinical skin infections [39,167]. Indeed, using the highly variable superantigens as strain-specific indicator antigens, our group demonstrated that the antibody response in healthy carriers is highly specific for their colonizing strain [167]. In line with this, patients with failure of skin barriers, such as epidermolysis bullosa, are colonized with *S. aureus* at very high density and have unusually high amounts of anti-*S. aureus* antibodies in their bodily fluids. In spite of their chronic skin wounds, these patients rarely develop life-threatening systemic infection [169,170]. This supports the notion of adaptive immune protection.

Since antibody responses to *S. aureus* are partially strain-specific and *S. aureus* strains differ greatly in their repertoire of secreted antigens, Kolata *et al.* used a personalized approach to analyze the antibody response during *S. aureus* bacteremia. Using 2D-gel-based immunoproteomics the prospective study revealed that *S. aureus* carriers had established a specific IgG response to their colonizing *S. aureus* isolate already before infection onset [158]. In the case of bacteremia with their own endogenous strain, this pre-existing memory response was boosted, and IgG titers increased [158]. In contrast, non-carriers infected with an exogenous *S. aureus* strain, started from lower basal antibody levels, presumably because the immune systems had not previously been exposed to the invasive *S. aureus* isolate. Over the course of bacteremia, their antibody patterns were drastically altered with the acquisition of many new specificities and increases of titers [158]. An immunoproteome signature of 11 conserved *S. aureus* proteins was defined, and the proteins were recognized by antibodies in at least half of the bacteremic patients [158]. Using a similar approach, immunoproteome signatures of *S. aureus* colonization, skin and soft tissue infection, and bacteremia have been reported by Liew and colleagues [135]. Further studies of well-defined patient cohorts are urgently required to identify disease-specific immunoproteome signatures with diagnostic potential.

Prospective clinical studies may also reveal immune parameters predictive of disease outcome, as shown in a pilot study. By combining two immunoproteomic assays, *i.e.*, automated 1D-immunoblots and suspension arrays, *S. aureus* bacteremia patients could be stratified according to their risk of developing sepsis, and IgG specificities that can serve as a marker for protection from sepsis were identified [133]. Hence, the immunological “starting position,” seems to be important for disease outcome, which encourages efforts in vaccine design.

### 3.4.3. T-cell Responses to *S. aureus*

Compared to antibodies, less is known about the specificity of *S. aureus*-reactive T-cells. The cellular arm of the adaptive immune system merits attention, because effector T-cells decisively influence the innate and the adaptive immune response: Depending on their differentiation into helper T-cell subpopulations such as Th1, Th2, Th17 or Treg cells, T-cells shape the Ig (sub)class composition, support the formation of memory B-cells, and/or enforce the recruitment of neutrophils [171,172]. The broad repertoire of *S. aureus*-specific B-cells points to a large pool of *S. aureus*-specific T-cells, because most B-cell responses rely on T-cell help [38]. Moreover, certain subsets of T-cells are required for an efficient and fast clearance of invading *S. aureus* [173,174]. In particular, Th17 cells were found to play a protective role in different animal models of *S. aureus* infection, most prominently in cutaneous infection [175–179]. Additionally, the current knowledge about intracellularly persisting staphylococci suggests that *S. aureus*-specific CD8+ T-cells may play a role in the adaptive immune response to *S. aureus* as well [180].

The comprehensive analysis of the T-cell antigen repertoire of *S. aureus* is a challenging task for several reasons: First, antigen-specific T-cell activation has stringent requirements involving antigen processing and presentation by host cells. Second, *S. aureus* releases virulence factors that interfere with standard T-cell activation assays; superantigens activate large subpopulations of T-cells independent of their antigen specificity and override the antigen-specific responses in cell culture [181,182]. Moreover, toxins such as α-toxin or leukocidins kill T-cells [183,184]. Hence,
T-cell immunoproteomics has to build on prior knowledge about putative immunogenic antigens derived either from genome information or from the analysis of the antibody response. The specific T-cell repertoire can then be probed with recombinant *S. aureus* proteins. Applying this approach to the analysis of ten healthy adults, the *S. aureus*–specific T-cell pool was estimated to comprise 3.6% of the peripheral blood T.cells with an astounding 35-fold difference between individuals (range 0.2%–5.7%) [152]. The *S. aureus* antigen-reactive memory T-cells will probably influence the course of *S. aureus* infection.

Other comprehensive methods to identify T-cell epitopes include MHC microarrays and in-silico analyses. MHC microarrays use peptide-MHC complexes in combination with co-stimulatory molecules as probes and T-cell populations as targets and can map MHC-restricted T-cell epitopes [185]. However, such techniques have not been applied to the *S. aureus* T-cell immunome yet.

### 3.5. Metabolomics

Metabolome analyses comprehensively characterize the low molecular metabolites (<1 kDa), which occur in a cell in an impressive number (several thousand different molecular species) and chemical diversity. The two most important detection methods are MS and nuclear magnetic resonance (NMR) spectroscopy (Table 1) [186]. NMR spectroscopy is “non-destructive,” *i.e.*, biosamples such as urine or blood plasma can be analyzed without further sample preparation. However, it is relatively insensitive. For lower concentrations, mass spectrometry is the method of choice. It is often combined with different separation techniques such as gas chromatography, liquid chromatography and capillary electrophoresis. Substrate identification and absolute quantification is carried out by a comparison with mass spectral fingerprint libraries and reference standards.

There are a number of reasons why the metabolome is relevant for studying adaptive immune responses to *S. aureus* and for vaccine development. Firstly, metabolic processes in *S. aureus* are linked to virulence and invasive capabilities [186]. Secondly, the elucidation of the *in vivo* metabolism of *S. aureus* can lead to the identification of new antimicrobial targets and compounds [112]. Finally, there is evidence that the metabolic state of the host influences the adaptive immune response [187].

Metabolomics is a young research field. First studies show the potential of these technologies [112,188–191]. For example, Krismer *et al.* used a combined metabolomics and transcriptomics approach to explore the adaptation of *S. aureus* during colonization of the human nose [112]. They observed that the methionine biosynthetic pathway is strongly upregulated and hence represents an interesting antimicrobial target.

Particularly promising, but technically very demanding, is the direct analysis of samples from infected hosts (*in vivo* metabolomics). The metabolic profiles of bodily fluids (serum, urine) are expected to reflect the molecules generated by the host immune response and non-immune cells that are directly affected by the disease, as well as by the pathogen [192,193]. Hence, metabolome studies will not only help to understand host pathogen interaction during infection, but can also aid in finding novel approaches for diagnosis and treatment, including potential vaccine targets.

### 4. Omics Technologies in *S. aureus* Vaccine Development

The omics revolution, including novel bioinformatics tools for data analysis, has extended the options in vaccine research beyond empirical strategies, promising to speed up vaccine development. It enables an approach termed “reverse vaccinology,” a genome-based unbiased discovery process for candidate vaccine antigens (Figure 2) [194–197]. Rather than starting from live attenuated or inactivated microorganisms or drawing on prior knowledge about pathogen-host interaction, reverse vaccinology begins with an analysis of the microbial genome for open reading frames to reveal the putative proteome. This analysis can be refined in several ways to narrow down the number of candidate antigens to be tested in pre-clinical models: (1) Computational comparison will reveal the degree of protein conservation within and between microbial species; (2) tools predicting subcellular localization can be used for filtering out molecules accessible to antibodies, namely, proteins released
by the microorganism or expressed on its surface; and (3) there are algorithms predicting T-cell and B-cell epitopes and hence immunogenicity [197–202]. Recombinant expression of the in-silico selected vaccine candidates and testing them for immunogenicity and protection in pre-clinical models are then the next steps (Figure 2) [194].

**Figure 2.** Combined approaches to successful *S. aureus* vaccine development. In empirical vaccinology, infection-relevant *S. aureus* antigens can be comprehensively mapped using transcriptomics and proteomics. In parallel, immunoproteomics provides a panoramic view of the intensity and dynamics of antibody binding to *S. aureus* proteins revealing their immunogenicity. Moreover, omics technologies can aid the characterization of the T-cell response to *S. aureus* in its full complexity. These empirical approaches will lead to the discovery of promising *S. aureus* vaccine candidates. Reverse vaccinology is a genome-based unbiased discovery process for candidate vaccine antigens. First, the whole *S. aureus* genome is mined for potential B- and T-cell epitopes using computer-based algorithms. Next, candidate antigens are produced as recombinant proteins and purified. These antigens as well as those that have been identified empirically are then used for vaccination in pre-clinical infection models and assayed for their ability to mediate protection. Promising candidate vaccines will then be subjected to clinical trials (not shown). Hence, omics technologies are versatile tools empowering both empirical and in-silico-based vaccine development.

With the intention of further reducing the number of microbial antigens to be examined, computer aided selection tools have been developed that draw on available information about successful vaccine antigens to deduce common features, such as chemical properties of amino acid sequences [203] or functional domains [204], and apply this knowledge to the discovery of vaccine candidates in microbial genome databases. The latter approach, termed protectome analysis, is based on the notion that protective vaccines should target bacterial virulence factors that are dangerous for the host and hence share biological functions in addition to being immunogenic [204].
In a pilot study, Oprea et al. employed a simplified reverse vaccinology approach to identify *S. aureus* candidate epitopes that induce both B- and T-cell mediated immunity [205]. Instead of starting with the whole bacterial genome, they selected ten conserved surface exposed proteins for antigenicity testing and identified epitopes from fibronectin binding protein A (FnbpA), collagen adhesion (Cna), serine-rich adhesin for platelets (SraP) and elastine binding protein (EbpS) as putative targets. These, however, still need to be validated [205].

On the other hand, omics technologies are also empowering empirical approaches to vaccine development (Figure 2). They can be applied to the study of preclinical models but also of vaccine target populations directly. Given the fact that successful vaccination in mice could not yet be translated into an effective human vaccine, this is a big advantage. As shown above, the natural human immune response to *S. aureus* colonization and infection can now be mapped with unprecedented completeness and resolution [157]. Immunoproteomics provides a panoramic view of the intensity and dynamics of antibody binding to *S. aureus* proteins revealing their immunogenicity [40,158,206,207]. Antibody profiling in patient cohorts by multiplex assays is useful for hypothesis testing as well as for hypothesis generation [133,169,208–210]. It leads to the discovery of promising vaccine candidates such as the small set of *S. aureus* antigens, whose recognition by antibodies was associated with protection from sepsis in *S. aureus* bacteremia patients [133]. Finally, antibody binding is a good lead for T-cell antigen selection, since the development of high affinity antibodies requires T-cell help in most cases [152]. For targeting *S. aureus* persisting inside host cells comprehensive information about the transcription profile of the bacteria as well as the proteome produced by them is invaluable [95,139,144].

Combining empirical and *in-silico* strategies will enable vaccine researchers to benefit from all available information. Genome-based *in-silico* methods in conjunction with proteomics, for example, enable the discovery of cryptic proteins that do not elicit a prominent natural immune response but can nevertheless serve an important role in host-pathogen interaction. Moreover, T-cells may be activated by intra-cellular bacterial proteins, which are not accessible to B-cells and therefore do not elicit an antibody response. The search for such T-cell antigens may be aided by computational approaches. On the other hand, the prediction tools of reverse vaccinology need to be empirically validated. While it may be difficult and of little appeal to test proteins in preclinical models that are anticipated to be useless, immunoproteomics of naturally colonized or infected humans or animals can also serve to test a number of predictions made by the sophisticated bioinformatics tools that are now available to the research community.

5. Future Directions of Research

Omics techniques are developing at a breathtaking pace holding promise for both hypothesis generation and hypothesis testing. The following issues should be addressed in order to apply their full potential to the elucidation of *S. aureus*-host interaction, especially the adaptive immune response, and to vaccine development:

1. Improvement of important aspects of pre-analytics, such as the rapid enrichment of the pathogen from infected cells and tissues, as well as further increases of the sensitivity of detection methods, should ultimately permit the analysis of pathogen-host interactions directly *ex vivo*.

2. Interpretation of the “big data” generated by omics techniques relies on sophisticated bioinformatics and depends on the inter-disciplinary dialogue as well as on innovation and technical optimization in the field of computational statistics and bioinformatics.

3. Prospective clinical trials in well-defined patient cohorts will remain key to finding answers to the burning questions at hand. Such clinical studies should simultaneously consider the pathogen and the immune response, collecting bacterial strains as well as patient sera and immune cells.

4. Disease specific transcriptome and proteome profiles are required to explain the broad spectrum of diseases caused by the versatile species *S. aureus* and to develop targeted counter-measures.
5) Advanced omics technologies should be applied to study the adaptive immune response to *S. aureus*. Mapping of antigen, antibody and T-cell repertoires may reveal correlates of protection on which vaccination strategies can then be based.

6) The multi-omics-approach is very much focused on genes and proteins. Non-protein molecules, however, may be equally important in *S. aureus*-host interaction.

**Acknowledgments:** The authors would like to thank Holger Kock for providing information on omics technologies. Funding: Deutsche Forschungsgemeinschaft/German Research Foundation (CRC-TRR34, RTG1870), German Ministry for Education and Research (HICARE, InfectControl2020).

**Conflicts of Interest:** The authors declare no conflict of interest.

**References**

1. Wertheim, H.F.; Melles, D.C.; Vos, M.C.; van Leeuwen, W.; van Belkum, A.; Verbrugh, H.A.; Nouwen, J.L. The role of nasal carriage in *Staphylococcus aureus* infections. *Lancet Infect. Dis.* 2005, 5, 751–762. [CrossRef]
2. Lowy, F.D. Staphylococcus aureus Infections. *N. Engl. J. Med.* 1998, 339, 520–532. [CrossRef] [PubMed]
3. Hidron, A.I.; Edwards, J.R.; Patel, J.; Horan, T.C.; Sievert, D.M.; Pollock, D.A.; Fridkin, S.K.; National Healthcare Safety Network Team; Participating National Healthcare Safety Network Facilities. NHSN annual update: Antimicrobial-resistant pathogens associated with healthcare-associated infections: Annual summary of data reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention, 2006–2007. *Infect. Control Hosp. Epidemiol.* 2008, 29, 996–1011. [CrossRef] [PubMed]
4. Klevens, R.M.; Morrison, M.A.; Nadle, J.; Petit, S.; Gershman, K.; Ray, S.; Harrison, L.H.; Lynfield, R.; Dumyati, G.; Townes, J.M.; *et al.* Invasive methicillin-resistant *Staphylococcus aureus* infections in the United States. *JAMA* 2007, 298, 1763–1771. [CrossRef] [PubMed]
5. Jones, R.N. Microbial etiologies of hospital-acquired bacterial pneumonia and ventilator-associated bacterial pneumonia. *Clin. Infect. Dis.* 2010, 51 (Suppl. 1), S81–S87. [CrossRef] [PubMed]
6. David, M.Z.; Daum, R.S. Community-associated methicillin-resistant *Staphylococcus aureus*: Epidemiology and clinical consequences of an emerging epidemic. *Clin. Microbiol. Rev.* 2010, 23, 616–687. [CrossRef] [PubMed]
7. De Kraker, M.E.; Wolkewitz, M.; Davey, P.G.; Koller, W.; Berger, J.; Nagler, J.; Icket, C.; Kalenic, S.; Horvatic, J.; Seifert, H.; *et al.* Clinical impact of antimicrobial resistance in European hospitals: Excess mortality and length of hospital stay related to methicillin-resistant *Staphylococcus aureus* bloodstream infections. *Antimicrob. Agents Chemother.* 2011, 55, 1598–1605. [CrossRef] [PubMed]
8. Cosgrove, S.E.; Sakoulas, G.; Perencevich, E.N.; Schaeber, M.J.; Karchmer, A.W.; Carmeli, Y. Comparison of mortality associated with methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* bacteremia: A meta-analysis. *Clin. Infect. Dis.* 2003, 36, 53–59. [CrossRef] [PubMed]
9. Boucher, H.W.; Corey, G.R. Epidemiology of Methicillin-Resistant *Staphylococcus aureus*. *Clin. Infect. Dis.* 2008, 46, S344–S349. [CrossRef] [PubMed]
10. World Health Organization. *Antimicrobial Resistance: Global Report on Surveillance*; World Health Organization: Geneva, Switzerland, 2014.
11. Daum, R.S.; Spellberg, B. Progress Toward a *Staphylococcus aureus* Vaccine. *Clin. Infect. Dis.* 2012, 54, 560–567. [CrossRef] [PubMed]
12. Proctor, R.A. Challenges for a universal *Staphylococcus aureus* vaccine. *Clin. Infect. Dis.* 2012, 54, 1179–1186. [CrossRef] [PubMed]
13. Fowler, V.G., Jr.; Proctor, R.A. Where does a *Staphylococcus aureus* vaccine stand? *Clin. Microbiol. Infect.* 2014, 20 (Suppl. 5), 66–75. [CrossRef] [PubMed]
14. Fleischmann, R.D.; Adams, M.D.; White, O.; Clayton, R.A.; Kirkness, E.F.; Kerlavage, A.R.; Bult, C.J.; Tomb, J.F.; Dougherty, B.A.; Merrick, J.M.; *et al.* Whole-genome random sequencing and assembly of *Haemophilus influenzae Rd*. *Science* 1995, 269, 496–512. [CrossRef] [PubMed]
15. Lander, E.S.; Linton, L.M.; Birren, B.; Nusbaum, C.; Zody, M.C.; Baldwin, J.; Devon, K.; Dewar, K.; Doyle, M.; FitzHugh, W.; *et al.* Initial sequencing and analysis of the human genome. *Nature* 2001, 409, 860–921. [CrossRef] [PubMed]
16. Venter, J.C.; Adams, M.D.; Myers, E.W.; Li, P.W.; Mural, R.J.; Sutton, G.G.; Smith, H.O.; Yandell, M.; Evans, C.A.; Holt, R.A.; et al. The sequence of the human genome. *Science* 2001, 291, 1304–1351. [CrossRef] [PubMed]

17. Van Belkum, A.; Verkaik, N.J.; de Vogel, C.P.; Boelens, H.A.; Verveer, J.; Nouwen, J.L.; Verbrugh, H.A.; Wertheim, H.F. Reclassification of *Staphylococcus aureus* nasal carriage types. *J. Infect. Dis.* 2009, 15, 1820–1826. [CrossRef] [PubMed]

18. Wertheim, H.F.; Vos, M.C.; Ott, A.; van Belkum, A.; Voss, A.; Kuytmans, J.A.; van Keulen, P.H.; Vandenbroucke-Grauls, C.M.; Meester, M.H.; Verbrugh, H.A. Risk and outcome of nosocomial *Staphylococcus aureus* bacteraemia in nasal carriers versus non-carriers. *Lancet* 2004, 364, 703–705. [CrossRef]

19. Von Eiff, C.; Becker, K.; Machka, K.; Stammer, H.; Peters, G. Nasal Carriage as a Source of *Staphylococcus aureus* Bacteremia. *N. Engl. J. Med.* 2001, 344, 11–16. [CrossRef] [PubMed]

20. Wertheim, H.F.; Vos, M.C.; Ott, A.; Voss, A.; Kuytmans, J.A.; Vandenbroucke-Grauls, C.M.; Meester, M.H.; van Keulen, P.H.; Verbrugh, H.A. Mupirocin prophylaxis against nosocomial *Staphylococcus aureus* infections in nonsurgical patients: A randomized study. *Ann. Intern. Med.* 2004, 140, 419–425. [CrossRef]

21. Bode, L.G.; Kuytmans, J.A.; Wertheim, H.F.; Bogaers, D.; Vandenbroucke-Grauls, C.M.; Roosendaal, R.; Troelstra, A.; Box, A.T.; Voss, A.; van der Tweel, I.; et al. Preventing surgical-site infections in nasal carriers of *Staphylococcus aureus*. *N. Engl. J. Med.* 2010, 362, 9–17. [CrossRef] [PubMed]

22. Löffler, B.; Tuchscherr, L.; Niemann, S.; Peters, G. *Staphylococcus aureus* persistence in non-professional phagocytes. *Int. J. Med. Microbiol.* 2014, 304, 170–176. [CrossRef] [PubMed]

23. Tuchscherr, L.; Medina, E.; Hussain, M.; Volker, W.; Heitmann, V.; Niemann, S.; Holzinger, D.; Roth, J.; Proctor, R.A.; Becker, K.; et al. Staphylococcus aureus phenotype switching: An effective bacterial strategy to escape host immune response and establish a chronic infection. *EMBO Mol. Med.* 2011, 3, 129–141. [CrossRef] [PubMed]

24. Lindsay, J.A.; Holden, M.T. Staphylococcus aureus: Superbug, super genome? *Trends Microbiol.* 2004, 12, 378–385. [CrossRef] [PubMed]

25. Lindsay, J.A.; Moore, C.E.; Day, N.P.; Peacock, S.J.; Witney, A.A.; Stabler, R.A.; Husain, S.E.; Butcher, P.D.; Hinds, J. Microarrays Reveal that Each of the Ten Dominant Lineages of *Staphylococcus aureus* Has a Unique Combination of Surface-Associated and Regulatory Genes. *J. Bacteriol.* 2006, 188, 669–676. [CrossRef] [PubMed]

26. Stranger-Jones, Y.K.; Bae, T.; Schneewind, O. Vaccine assembly from surface proteins of *Staphylococcus aureus*. *Proc. Natl. Acad. Sci. USA* 2006, 103, 16942–16947. [CrossRef] [PubMed]

27. Zuo, Q.F.; Yang, L.Y.; Feng, Q.; Lu, D.S.; Dong, Y.D.; Cai, C.Z.; Wu, Y.; Guo, Y.; Gu, J.; Zeng, H.; et al. Evaluation of the protective immunity of a novel subunit fusion vaccine in a murine model of systemic MRSA infection. *PLoS ONE* 2013, 8, e81212. [CrossRef] [PubMed]

28. Spaulding, A.R.; Salgado-Pabon, W.; Merriiman, J.A.; Stach, C.S.; Ji, Y.; Gillman, A.N.; Peterson, M.L.; Schlievert, P.M. Vaccination against *Staphylococcus aureus* pneumonia. *J. Infect. Dis.* 2014, 209, 1955–1962. [CrossRef] [PubMed]

29. Burian, M.; Rautenberg, M.; Kohler, T.; Fritz, M.; Krismer, B.; Unger, C.; Hoffmann, W.H.; Peschel, A.; Wolz, C.; Goerke, C. Temporal expression of adhesion factors and activity of global regulators during establishment of *Staphylococcus aureus* nasal colonization. *J. Infect. Dis.* 2010, 201, 1414–1421. [CrossRef] [PubMed]

30. Yarwood, J.M.; McCormick, J.K.; Paustian, M.L.; Kapur, V.; Schlievert, P.M. Repression of the *Staphylococcus aureus* Accessory Gene Regulator in Serum and In Vivo. *J. Bacteriol.* 2002, 184, 1095–1101. [CrossRef] [PubMed]

31. Pragman, A.A.; Schlievert, P.M. Virulence regulation in *Staphylococcus aureus*: The need for in vivo analysis of virulence factor regulation. *FEMS Immunol. Med. Microbiol.* 2004, 42, 147–154. [CrossRef] [PubMed]

32. Hanses, F.; Roux, C.; Dunman, P.M.; Salzberger, B.; Lee, J.C. *Staphylococcus aureus* gene expression in a rat model of infective endocarditis. *Genome Med.* 2014, 6, 93. [CrossRef] [PubMed]

33. Löffler, B.; Hussain, M.; Grundmeier, M.; Bruck, M.; Holzinger, D.; Varga, G.; Roth, J.; Kahl, B.C.; Proctor, R.A.; Peters, G. *Staphylococcus aureus* panton-valentine leukocidin is a very potent cytotoxic factor for human neutrophils. *PLoS Pathog.* 2010, 6, e1000715. [CrossRef] [PubMed]
34. Rooijakkers, S.H.; Ruyken, M.; Roos, A.; Daha, M.R.; Presanis, J.S.; Sim, R.B.; van Wamel, W.J.; van Kessel, K.P.; van Strijp, J.A. Immune evasion by a staphylococcal complement inhibitor that acts on C3 convertases. *Nat. Immunol.* 2005, 6, 920–927. [CrossRef] [PubMed]
35. Rooijakkers, S.H.; van Kessel, K.P.; van Strijp, J.A. Staphylococcal innate immune evasion. *Trends Microbiol.* 2005, 13, 596–601. [CrossRef] [PubMed]
36. Langley, R.; Wines, B.; Willoughby, N.; Basu, I.; Proft, T.; Fraser, J.D. The staphylococcal superantigen-like protein 7 binds IgA and complement C5 and inhibits IgA-Fc alpha RI binding and serum killing of bacteria. *J. Immunol.* 2005, 174, 2926–2933. [CrossRef] [PubMed]
37. Salgado-Pabon, W.; Schlievert, P.M. Models matter: The search for an effective Staphylococcus aureus vaccine. *Nat. Rev. Microbiol.* 2014, 12, 585–591. [CrossRef] [PubMed]
38. Broker, B.M.; Holtfreter, S.; Bekerded-Jing, I. Immune control of Staphylococcus aureus—Regulation and counter-regulation of the adaptive immune response. *Int. J. Med. Microbiol.* 2014, 304, 204–214. [CrossRef] [PubMed]
39. Holtfreter, S.; Kolata, J.; Broker, B.M. Towards the immune proteome of Staphylococcus aureus—The anti-*S. aureus* antibody response. *Int. J. Med. Microbiol.* 2010, 300, 176–192. [CrossRef] [PubMed]
40. Holtfreter, S.; Nguyen, T.T.; Wertheim, H.; Steil, L.; Kusch, H.; Truong, Q.P.; Engelmann, S.; Hecker, M.; Volker, U.; van Belkum, A.; et al. Human immune proteome in experimental colonization with Staphylococcus aureus. *Clin. Vaccine Immunol.* 2009, 16, 1607–1614. [CrossRef] [PubMed]
41. Verkaik, N.J.; de Vogel, C.P.; Boelens, H.A.; Grumann, D.; Hoogenboezem, T.; Vink, C.; Hooijkaas, H.; Foster, T.J.; Verbrugh, H.A.; van Belkum, A.; et al. Anti-staphylococcal humoral immune response in persistent nasal carriers and noncarriers of Staphylococcus aureus. *J. Infect. Dis.* 2009, 199, 625–632. [CrossRef] [PubMed]
42. Öie, S.; Kamiya, A. Survival of methicillin-resistant Staphylococcus aureus (MRSA) on naturally contaminated dry mops. *J. Hosp. Infect.* 1996, 34, 145–149. [CrossRef]
43. O’Connell, N.H.; Humphreys, H. Intensive care unit design and environmental factors in the acquisition of infection. *J. Hosp. Infect.* 2000, 45, 255–262. [CrossRef] [PubMed]
44. Koser, C.U.; Holden, M.T.; Ellington, M.J.; Cartwright, E.J.; Brown, N.M.; Ogilvy-Stuart, A.L.; Hsu, L.Y.; Chewapreecha, C.; Croucher, N.J.; Harris, S.R.; et al. Rapid whole-genome sequencing for investigation of a neonatal MRSA outbreak. *N. Engl. J. Med.* 2012, 366, 2267–2275. [CrossRef] [PubMed]
45. Paterson, G.K.; Harrison, E.M.; Murray, G.G.; Han, Q.P.; Engelmann, S.; Hecker, M.; Volker, U.; van Belkum, A.; et al. Human immune proteome in experimental colonization with Staphylococcus aureus. *Clin. Vaccine Immunol.* 2009, 16, 1607–1614. [CrossRef] [PubMed]
46. Laabei, M.; Recker, M.; Rudkin, J.K.; Aldeljawi, M.; Gulay, Z.; Sloan, T.J.; Williams, P.; Endres, J.L.; Bayles, K.W.; Fey, P.D.; et al. Predicting the virulence of MRSA from its genome sequence. *Genome Res.* 2014, 24, 839–849. [CrossRef] [PubMed]
47. Holden, M.T.; Hsu, L.Y.; Kurt, I.; Weinert, L.A.; Mather, A.E.; Harris, S.R.; Strommenger, B.; Layer, F.; Witte, W.; de Lencastre, H.; et al. A genomic portrait of the emergence, evolution, and global spread of a methicillin-resistant Staphylococcus aureus pandemic. *Genome Res.* 2013, 23, 653–664. [CrossRef] [PubMed]
48. Harris, S.R.; Feil, E.J.; Holden, M.T.; Quail, M.A.; Nickerson, E.K.; Chantratita, N.; Gardete, S.; Tavares, A.; Day, N.; Lindsay, J.A.; et al. Evolution of MRSA during hospital transmission and intercontinental spread. *Science* 2010, 327, 469–474. [CrossRef] [PubMed]
49. DeLeo, F.R.; Kennedy, A.D.; Chen, L.; Wardenburg, J.B.; Kobayashi, S.D.; Mathema, B.; Braughton, K.R.; Whitney, A.R.; Villaruz, A.E.; Martens, C.A.; et al. Molecular differentiation of historic phage-type 80/81 and contemporary epidemic Staphylococcus aureus. *Proc. Natl. Acad. Sci. USA* 2011, 108, 18091–18096. [CrossRef] [PubMed]
50. Kurt, K.; Rasigade, J.P.; Laurent, F.; Goering, R.V.; Zemlickova, H.; Machova, I.; Struelens, M.J.; Zautner, A.E.; Holtfreter, S.; Bröker, B.; et al. Subpopulations of *Staphylococcus aureus* clonal complex 121 are associated with distinct clinical entities. *PLoS ONE* 2013, 8, e58155. [CrossRef] [PubMed]
51. McAdam, P.R.; Templeton, K.E.; Edwards, G.F.; Holden, M.T.; Feil, E.J.; Aanensen, D.M.; Bargawi, H.J.; Spratt, B.G.; Bentley, S.D.; Parkhill, J.; et al. Molecular tracing of the emergence, adaptation, and transmission of hospital-associated methicillin-resistant Staphylococcus aureus. *Proc. Natl. Acad. Sci. USA* 2012, 109, 9107–9112. [CrossRef] [PubMed]
52. Luedicke, C.; Slickers, P.; Ehrricht, R.; Monecke, S. Molecular fingerprinting of Staphylococcus aureus from bone and joint infections. *Eur. J. Clin. Microbiol. Infect. Dis.* 2010, 29, 457–463. [CrossRef] [PubMed]

53. Monecke, S.; Slickers, P.; Hotzel, H.; Richter-Huhn, G.; Pohle, M.; Weber, S.; Witte, W.; Ehrricht, R. Microarray-based characterisation of a Panton-Valentine leukocidin-positive community-acquired strain of methicillin-resistant Staphylococcus aureus. *Clin. Microbiol. Infect.* 2006, 12, 718–728. [CrossRef] [PubMed]

54. Monecke, S.; Luedicke, C.; Slickers, P.; Ehrricht, R. Molecular epidemiology of Staphylococcus aureus in asymptomatic carriers. *Eur. J. Clin. Microbiol. Infect. Dis.* 2009, 28, 1159–1165. [CrossRef] [PubMed]

55. Masoud-Landgraf, L.; Johler, S.; Badura, A.; Feierl, G.; Luxner, J.; Wagner-Eibl, U.; Eber, E.; Zarfel, G.; Grisold, A.J. Genetic and Phenotypic Characteristics of Staphylococcus aureus Isolates from Cystic Fibrosis Patients in Austria. *Respir. Int. Rev. Thorac. Dis.* 2015, 89, 390–395. [CrossRef] [PubMed]

56. Monecke, S.; Ehrricht, R. Rapid genotyping of methicillin-resistant Staphylococcus aureus (MRSA) isolates using miniaturised oligonucleotide arrays. *Clin. Microbiol. Infect.* 2005, 11, 825–833. [CrossRef] [PubMed]

57. Dunman, P.M.; Murphy, E.; Haney, S.; Palacios, D.; Tucker-Kellogg, G.; Wu, S.; Brown, E.L.; Zagursky, R.J.; Shlaes, D.; Projan, S.J. Transcription profiling-based identification of *Staphylococcus aureus* genes regulated by the agr and/or sarA loci. *J. Bacteriol.* 2001, 183, 7341–7353. [CrossRef] [PubMed]

58. Witney, A.A.; Marsden, G.L.; Holden, M.T.; Stabler, R.A.; Husain, S.E.; Vass, J.K.; Butcher, P.D.; Hinds, J.; Lindsay, J.A. Design, validation, and application of a seven-strain Staphylococcus aureus PCR product microarray for comparative genomics. *Appl. Environ. Microbiol.* 2005, 71, 7504–7514. [CrossRef] [PubMed]

59. McManus, B.A.; Coleman, D.C.; Deasy, E.C.; Brennan, G.I.; O’Connell, B.; Monecke, S.; Ehrricht, R.; Leggett, B.; Leonard, N.; Shore, A.C. Comparative Genotypes, Staphylococcal Cassette Chromosome mec (SCCmec) Genes and Antimicrobial Resistance amongst Staphylococcus epidermidis and Staphylococcus haemolyticus Isolates from Infections in Humans and Companion Animals. *PLoS ONE* 2015, 10, e0138079. [CrossRef] [PubMed]

60. Thunberg, U.; Hugosson, S.; Monecke, S.; Ehrricht, R.; Soderquist, B. Molecular characteristics of Staphylococcus aureus associated with chronic rhinosinusitis. *APMIS* 2015, 123, 37–44. [CrossRef] [PubMed]

61. Rasmussen, G.; Monecke, S.; Brus, O.; Ehrricht, R.; Soderquist, B. Long term molecular epidemiology of methicillin-susceptible Staphylococcus aureus bacteremia isolates in Sweden. *PLoS ONE* 2014, 9, e114276. [CrossRef] [PubMed]

62. Shore, A.C.; Brennan, O.M.; Deasy, E.C.; Rossney, A.S.; Kinnevey, P.M.; Ehrricht, R.; Monecke, S.; Coleman, D.C. DNA microarray profiling of a diverse collection of nosocomial methicillin-resistant staphylococcus aureus isolates assigns the majority to the correct sequence type and staphylococcal cassette chromosome mec (SCCmec) type and results in the subsequent identification and characterization of novel SCCmec-SCCM1 composite islands. *Antimicrob. Agents Chemother.* 2012, 56, 5340–5355. [PubMed]

63. Monecke, S.; Engelmann, I.; Archambault, M.; Coleman, D.C.; Coombs, G.W.; Cortez de Jackel, S.; Pelletier-Jacques, G.; Schwarz, S.; Shore, A.C.; Slickers, P.; et al. Distribution of SCCmec-associated phenol-soluble modulin in staphylococci. *Mol. Cell. Probes* 2012, 26, 99–103. [CrossRef] [PubMed]

64. Shukla, S.K.; Rose, W.; Schrodi, S.J. Complex host genetic susceptibility to Staphylococcus aureus infections. *Trends Microbiol.* 2015, 23, 529–536. [CrossRef] [PubMed]

65. Emonts, M.; Uitterlinden, A.G.; Nouwen, J.L.; Kardys, I.; Maat, M.P.; Melles, D.C.; Witteman, J.; Jong, P.T.; Verbrugh, H.A.; Hofman, A.; et al. Host polymorphisms in interleukin 4, complement factor H, and C-reactive protein associated with nasal carriage of Staphylococcus aureus and occurrence of boils. *J. Infect. Dis.* 2008, 197, 124–1253. [CrossRef] [PubMed]

66. Nurjadi, D.; Herrmann, E.; Hinderberger, I.; Zanger, P. Impaired beta-defensin expression in human skin links DEFB1 promoter polymorphisms with persistent Staphylococcus aureus nasal carriage. *J. Infect. Dis.* 2013, 207, 666–674. [CrossRef] [PubMed]

67. Ruimy, R.; Angebault, C.; Djossou, F.; Dupont, C.; Epelboin, L.; Jarraud, S.; Lefevre, L.A.; Bes, M.; Lixandrù, B.E.; Bertine, M.; et al. Are host genetics the predominant determinant of persistent nasal Staphylococcus aureus carriage in humans? *J. Infect. Dis.* 2010, 202, 924–934. [CrossRef] [PubMed]

68. van den Akker, E.L.; Nouwen, J.L.; Melles, D.C.; van Rossum, E.F.; Koper, J.W.; Uitterlinden, A.G.; Hofman, A.; Verbrugh, H.A.; Pols, H.A.; Lamberts, S.W.; et al. Staphylococcus aureus nasal carriage is associated with glucocorticoid receptor gene polymorphisms. *J. Infect. Dis.* 2006, 194, 814–818. [CrossRef] [PubMed]
69. Nelson, C.L.; Pelak, K.; Podgoreanu, M.V.; Ahn, S.H.; Scott, W.K.; Allen, A.S.; Cowell, L.G.; Rude, T.H.; Zhang, Y.; Tong, A.; et al. A genome-wide association study of variants associated with acquisition of Staphylococcus aureus bacteremia in a healthcare setting. *BMC Infect Dis* **2014**, *14*, 83. [CrossRef] [PubMed]

70. Ye, Z.; Vasco, D.A.; Carter, T.C.; Brilliant, M.H.; Schrodi, S.J.; Shukla, S.K. Genome wide association study of SNP-, gene-, and pathway-based approaches to identify genes influencing susceptibility to Staphylococcus aureus infections. *Front. Genet.* **2014**, *5*, 125. [CrossRef] [PubMed]

71. Cormier, C.; Mfuna Endam, L.; Filali-Mouhim, A.; Boisvert, P.; Boulet, L.P.; Boulay, M.E.; Vallee-Smedja, S.; Besgen, P.; Desrosiers, M. A pooling-based genomewide association study identifies genetic variants associated with Staphylococcus aureus colonization in chronic rhinosinusitis patients. *Int. Forum Allergy Rhinol.* **2014**, *4*, 207–215. [CrossRef] [PubMed]

72. Six, A.; Mariotti-Ferrandiz, M.E.; Chaara, W.; Magadan, S.; Pham, H.P.; Lefranc, M.P.; Mora, T.; Thomas-Vasin, V.; Walczak, A.M.; Boudinot, P. The past, present, and future of immune repertoire biology—The rise of next-generation repertoire analysis. *Front. Immunol.* **2013**, *4*, 413. [CrossRef] [PubMed]

73. Han, A.; Glenville, J.; Hansmann, L.; Davis, M.M. Linking T-cell receptor sequence to functional phenotype at the single-cell level. *Nat. Biotechnol.* **2014**, *32*, 684–692. [CrossRef] [PubMed]

74. Newell, E.W.; Davis, M.M. Beyond model antigens: High-dimensional methods for the analysis of antigen-specific T-cells. *Nat. Biotechnol.* **2014**, *32*, 149–157. [CrossRef] [PubMed]

75. Calis, J.J.; Rosenberg, B.R. Characterizing immune repertoires by high throughput sequencing: Strategies and applications. *Trends Immunol.* **2014**, *35*, 581–590. [CrossRef] [PubMed]

76. Li, D.; Gao, G.; Li, Z.; Sun, W.; Li, X.; Chen, N.; Sun, J.; Yang, Y. Profiling the T-cell receptor repertoire of patient with pleural tuberculosis by high-throughput sequencing. *Immunol. Lett.* **2014**, *162*, 170–180. [CrossRef] [PubMed]

77. Diluvio, L.; Vollmer, S.; Besgen, P.; Ellwart, J.W.; Chimenti, S.; Prinz, J.C. Identical TCR beta-chain rearrangements in streptococcal angina and skin lesions of patients with psoriasis vulgaris. *J. Immunol.* **2006**, *176*, 7104–7111. [CrossRef] [PubMed]

78. Westermann, A.J.; Forstner, K.U.; Amman, F.; Barquist, L.; Chao, Y.; Schulte, L.N.; Muller, L.; Reinhardt, R.; Stdler, P.F.; Vogel, J. Dual RNA-seq unveils noncoding RNA functions in host-pathogen interactions. *Nature* **2016**, *529*, 496–501. [CrossRef] [PubMed]

79. Westermann, A.J.; Gorski, S.A.; Vogel, J. Dual RNA-seq of pathogen and host. *Nat. Rev. Microbiol.* **2012**, *10*, 618–630. [CrossRef] [PubMed]

80. Humphrys, M.S.; Creasy, T.; Sun, Y.; Shetty, A.C.; Chibucos, M.C.; Drabek, E.F.; Fraser, C.M.; Farooq, U.; Sengamalay, N.; Ott, S.; et al. Simultaneous transcriptional profiling of bacteria and their host cells. *PLoS ONE* **2013**, *8*, e80597. [CrossRef] [PubMed]

81. Chaves-Moreno, D.; Wos-Oxley, M.L.; Jauregui, R.; Medina, E.; Oxley, A.P.A.; Pieper, D.H. Application of a Novel “Pan-Genome”-Based Strategy for Assigning RNASeq Transcript Reads to Staphylococcus aureus Strains. *PLoS ONE* **2015**, *10*, e0145861.

82. Cui, L.; Lian, J.Q.; Neoh, H.M.; Reyes, E.; Hiramatsu, K. DNA microarray-based identification of genes associated with glycopeptide resistance in Staphylococcus aureus. *Antimicrob. Agents Chemother.* **2005**, *49*, 3404–3413. [CrossRef] [PubMed]

83. Stevens, D.L.; Ma, Y.; Salmi, D.B.; McIndoo, E.; Wallace, R.J.; Bryant, A.E. Impact of antibiotics on expression of virulence-associated exotoxin genes in methicillin-sensitive and methicillin-resistant Staphylococcus aureus. *J. Infect. Dis.* **2007**, *195*, 202–211. [CrossRef] [PubMed]

84. Fischer, A.; Yang, S.J.; Bayer, A.S.; Vaezzadeh, A.R.; Herzig, S.; Stenz, L.; Girard, M.; Sakoulas, G.; Scherl, A.; Yeaman, M.R.; et al. Daptomycin resistance mechanisms in clinically derived Staphylococcus aureus strains assessed by a combined transcriptomics and proteomics approach. *J. Antimicrob. Chemother.* **2011**, *66*, 1696–1711. [CrossRef] [PubMed]

85. Reiss, S.; Pane-Farre, J.; Fuchs, S.; Francois, P.; Liebeke, M.; Schrenzel, J.; Lindequist, U.; Lalk, M.; Wolz, C.; Hecker, M.; et al. Global analysis of the Staphylococcus aureus response to mupirocin. *Antimicrob. Agents Chemother.* **2012**, *56*, 787–804. [CrossRef] [PubMed]

86. Palazzolo-Ballance, A.M.; Reniere, M.L.; Braughton, K.R.; Sturdevant, D.E.; Otto, M.; Kreiswirth, B.N.; Skarr, E.F.; DeLeo, F.R. Neutrophil microbicides induce a pathogen survival response in community-associated methicillin-resistant Staphylococcus aureus. *J. Immunol.* **2008**, *180*, 500–509. [CrossRef] [PubMed]
87. Chang, W.; Small, D.A.; Toghrul, F.; Bentley, W.E. Global transcriptome analysis of Staphylococcus aureus response to hydrogen peroxide. *J. Bacteriol.* 2006, 188, 1648–1659. [CrossRef] [PubMed]

88. Ishii, K.; Adachi, T.; Yasukawa, J.; Suzuki, Y.; Hamamoto, H.; Sekimizu, K. Induction of virulence gene expression in Staphylococcus aureus by pulmonary surfactant. *Infect. Immun.* 2014, 82, 1500–1510. [CrossRef] [PubMed]

89. Windmuller, N.; Witten, A.; Block, D.; Bunk, B.; Sproer, C.; Kahl, B.C.; Mellmann, A. Transcriptional adaptations during long-term persistence of Staphylococcus aureus in the airways of a cystic fibrosis patient. *Int. J. Med. Microbiol.* 2015, 305, 35–46. [CrossRef] [PubMed]

90. Santiago-Rodriguez, T.M.; Naidu, M.; Jones, M.B.; Ly, M.; Pride, D.T. Identification of staphylococcal phage with reduced transcription in human blood through transcriptome sequencing. *Front. Microbiol.* 2015, 6, 216. [CrossRef] [PubMed]

91. Oogai, Y.; Matsuo, M.; Hashimoto, M.; Kato, F.; Sugai, M.; Komatsuawa, H. Expression of virulence factors by Staphylococcus aureus grown in serum. *Appl. Environ. Microbiol.* 2011, 77, 8097–8105. [CrossRef] [PubMed]

92. Malachowa, N.; Kobayashi, S.D.; Sturdevant, D.E.; Scott, D.P.; DeLeo, F.R. Insights into the Staphylococcus aureus-host interface: Global changes in host and pathogen gene expression in a rabbit skin infection model. *PLoS ONE* 2015, 10, e0117713. [CrossRef] [PubMed]

93. Resch, A.; Rosenstein, R.; Nerz, C.; Gotz, F. Differential gene expression profiling of Staphylococcus aureus cultivated under biofilm and planktonic conditions. *Appl. Environ. Microbiol.* 2005, 71, 2663–2676. [CrossRef] [PubMed]

94. Seggewiss, J.; Becker, K.; Kotte, O.; Eisenacher, M.; Yazdi, M.R.; Fischer, A.; McNamara, P.; Al Laham, N.; Proctor, R.; Peters, G.; et al. Reporter metabolite analysis of transcriptional profiles of a Staphylococcus aureus strain with normal phenotype and its isogenic hemB mutant displaying the small-colony-variant phenotype. *J. Bacteriol.* 2006, 188, 7765–7777. [CrossRef] [PubMed]

95. Garzoni, C.; Francois, P.; Huyghe, A.; Couzinet, S.; Tapparel, C.; Charbonnier, Y.; Renzoni, A.; Lucchini, S.; Lew, D.P.; Vaudaux, P.; et al. A global view of *Staphylococcus aureus* whole genome expression upon internalization in human epithelial cells. *BMJ Genom.* 2007, 8, 171. [CrossRef] [PubMed]

96. Scherr, T.D.; Roux, C.M.; Hanke, M.L.; Angle, A.; Dunman, P.M.; Kielian, T. Global transcriptome analysis of Staphylococcus aureus biofilms in response to innate immune cells. *Infect. Immun.* 2013, 81, 4363–4376. [CrossRef] [PubMed]

97. Wang, X.; Xiu, L.; Hu, Q.; Cui, X.; Liu, B.; Tao, L.; Wang, T.; Wu, J.; Chen, Y.; Chen, Y. Deep sequencing-based transcriptional analysis of bovine mammary epithelial cells gene expression in response to in vitro infection with *Staphylococcus aureus* stains. *PLoS ONE* 2013, 8, e82117. [CrossRef] [PubMed]

98. Lewandowska-Sabat, A.M.; Boman, G.M.; Downing, A.; Talbot, R.; Storset, A.K.; Olsaker, I. The early phase transcriptome of bovine monocyte-derived macrophages infected with *Staphylococcus aureus* in vitro. *BMJ Genom.* 2013, 14, 891. [CrossRef] [PubMed]

99. Koziel, J.; Maciag-Gudowska, A.; Mikolajczyk, T.; Bzowska, M.; Sturdevant, D.E.; Whitney, A.R.; Shaw, L.N.; DeLeo, F.R.; Potempa, J. Phagocytosis of Staphylococcus aureus by macrophages exerts cytotoxic effects manifested by the upregulation of antiapoptotic factors. *PLoS ONE* 2009, 4, e5210. [CrossRef] [PubMed]

100. Kobayashi, S.D.; Braughton, K.R.; Palazzolo-Ballance, A.M.; Kennedy, A.D.; Sampaio, E.; Kristosturyan, E.; Whitney, A.R.; Sturdevant, D.E.; Dorward, D.W.; Holland, S.M.; et al. Rapid neutrophil destruction following phagocytosis of *Staphylococcus aureus*. *J. Innate Immun.* 2010, 2, 560–575. [CrossRef] [PubMed]

101. Toufeer, M.; Bonnefont, C.M.; Foulon, E.; Caubet, C.; Tasca, C.; Aurel, M.R.; Robert-Granie, C.; Rupp, R.; Foucaud, G. Gene expression profiling of dendritic cells reveals important mechanisms associated with predisposition to *Staphylococcus aureus* infections. *PLoS ONE* 2011, 6, e22147. [CrossRef] [PubMed]

102. Brady, R.A.; Bruno, V.M.; Burns, D.L. RNA-Seq Analysis of the Host Response to *Staphylococcus aureus* Skin and Soft Tissue Infection in a Mouse Model. *PLoS ONE* 2015, 10, e0124877. [CrossRef] [PubMed]

103. Li, R.; Zhang, C.L.; Liao, X.X.; Chen, D.; Wang, W.Q.; Zhu, Y.H.; Geng, X.H.; Ji, D.J.; Mao, Y.J.; Gong, Y.C.; et al. Transcriptome microRNA profiling of bovine mammary glands infected with *Staphylococcus aureus*. *Int. J. Mol. Sci.* 2015, 16, 4997–5013. [CrossRef] [PubMed]

104. Chen, J.; Feng, G.; Guo, Q.; Wardenburg, J.B.; Lin, S.; Inoshima, I.; Deaton, R.; Yuan, J.X.; Garcia, J.G.; Machado, R.F.; et al. Transcriptional events during the recovery from MRSA lung infection: A mouse pneumonia model. *PLoS ONE* 2013, 8, e70176. [CrossRef] [PubMed]
105. Depke, M.; Burian, M.; Schafer, T.; Broker, B.M.; Ohlsen, K.; Volker, U. The alternative sigma factor B modulates virulence gene expression in a murine Staphylococcus aureus infection model but does not influence kidney gene expression pattern of the host. *Int. J. Med. Microbiol.* 2012, 302, 33–39. [CrossRef] [PubMed]

106. Perez Novo, C.A.; Jedrzejczak-Czechowicz, M.; Lewandowska-Polak, A.; Claeys, C.; Holttappels, G.; Van Cauwenberge, P.; Kowalski, M.L.; Bachert, C. T-cell inflammatory response, Foxp3 and TNFRS18-L regulation of peripheral blood mononuclear cells from patients with nasal polyposis-asthma after staphylococcal superantigen stimulation. *Clin. Exp. Allergy* 2010, 40, 1323–1332. [CrossRef] [PubMed]

107. Niebuhr, M.; Scharonow, H.; Rathmann, M.; Mamerow, D.; Werfel, T. Staphylococcal exotoxins are strong inducers of IL-22: A potential role in atopic dermatitis. *J. Allergy Clin. Immunol.* 2010, 126, 1176–1183.e4. [CrossRef] [PubMed]

108. Matsu, K.; Nishikawa, A. Effects of the macrolide antibiotic, midecamycin, on Staphylococcus aureus product-induced Th2 cytokine response in patients with atopic dermatitis. *J. Interferon Cytokine Res.* 2004, 24, 197–201. [CrossRef] [PubMed]

109. Matsu, K.; Nishikawa, A. Percutaneous application of peptidoglycan from Staphylococcus aureus induces infiltration of CCR4+ cells into mouse skin. *J. Investig. Allergol. Clin. Immunol.* 2011, 21, 354–362. [PubMed]

110. Breuer, K.; Wittmann, M.; Kempe, K.; Kapp, A.; Mai, U.; Dittrich-Breiholz, O.; Kracht, M.; Mrabet-Dahbi, S.; Werfel, T. Alpha-toxin is produced by skin colonizing Staphylococcus aureus and induces a T helper type 1 response in atopic dermatitis. *Clin. Exp. Allergy* 2005, 35, 1088–1095. [CrossRef] [PubMed]

111. Burian, M.; Grummann, D.; Holtfreter, S.; Wolz, C.; Goerke, C.; Bröker, B.M. Expression of staphylococcal superantigens during nasal colonization is not sufficient to induce a systemic neutralizing antibody response in humans. *Eur. J. Clin. Microbiol. Infect. Dis.* 2012, 31, 251–256. [CrossRef] [PubMed]

112. Krismer, B.; Liebeke, M.; Janek, D.; Nega, M.; Rautenberg, M.; Hornig, G.; Unger, C.; Weidenmaier, C.; Otto, A.; Becher, D.; Schmidt, F.; Scharf, S.S.; Hildebrandt, P.; Dhople, V.H.; et al. High-resolution transcriptomic analysis of the adaptive response of staphylococcus aureus during acute and chronic phases of osteomyelitis. *mBio* 2014, 5, e01775-14. [CrossRef] [PubMed]

113. Chaffin, D.O.; Taylor, D.; Skerrett, S.J.; Rubens, C.E. Changes in the Staphylococcus aureus transcriptome during early adaptation to the lung. *PLoS ONE* 2012, 7, e41329. [CrossRef] [PubMed]

114. Szafranska, A.K.; Oxley, A.P.; Chaves-Moreno, D.; Horst, S.A.; Rosslenbroich, S.; Peters, G.; Goldmann, O.; Röhde, M.; Sinha, B.; Pieper, D.H.; et al. Global gene expression of methicillin-resistant Staphylococcus aureus USA300 during human and mouse infection. *J. Infect. Dis.* 2011, 204, 1542–1550. [CrossRef] [PubMed]

115. Weinrick, B.; Dunman, P.M.; McAleese, F.; Murphy, E.; Projan, S.J.; Fang, Y.; Novick, R.P. Effect of mild acid on gene expression in Staphylococcus aureus. *J. Bacteriol.* 2004, 186, 8407–8423. [CrossRef] [PubMed]

116. Maass, S.; Sievers, S.; Zuhlke, D.; Kuzinski, S.; Sappa, P.K.; Muntel, J.; Hessling, B.; Bernhardt, J.; Sietmann, R.; Volker, U.; et al. Efficient, global-scale quantification of absolute protein amounts by integration of targeted mass spectrometry and two-dimensional gel-based proteomics. *Anal. Chem.* 2011, 83, 2677–2684. [CrossRef] [PubMed]

117. Becher, D.; Hempel, K.; Sievers, S.; Zuhlke, D.; Pane-Farre, J.; Otto, A.; Fuchs, S.; Albrecht, D.; Bernhardt, J.; Engelmann, S.; et al. A proteomic view of an important human pathogen—towards the quantification of the entire Staphylococcus aureus proteome. *PLoS ONE* 2009, 4, e8176. [CrossRef] [PubMed]

118. Schmidt, F.; Scharf, S.S.; Hildebrandt, P.; Burian, M.; Bernhardt, J.; Dhople, V.; Kalinka, J.; Gutjahr, M.; Hammer, E.; Volker, U. Time-resolved quantitative proteome profiling of host-pathogen interactions: The response of Staphylococcus aureus RN1HG to internalisation by human airway epithelial cells. *Proteomics* 2010, 10, 2801–2811. [CrossRef] [PubMed]

119. Otto, A.; Becher, D.; Schmidt, F. Quantitative proteomics in the field of microbiology. *Proteomics* 2014, 14, 547–565. [CrossRef] [PubMed]

120. Otto, A.; Bernhardt, J.; Becker, M.; Becher, D. Global relative and absolute quantitation in microbial proteomics. *Curr. Opin. Microbiol.* 2012, 15, 364–372. [CrossRef] [PubMed]

121. Dreisbach, A.; van Dijl, J.M.; Buist, G. The cell surface proteome of Staphylococcus aureus. *Proteomics* 2011, 11, 3154–3168. [CrossRef] [PubMed]
123. Kusch, H.; Engelmann, S. Secrets of the secretome in Staphylococcus aureus. *Int. J. Med. Microbiol.* **2014**, *304*, 133–141. [CrossRef] [PubMed]

124. Depke, M.; Michalik, S.; Rabe, A.; Surmann, K.; Brinkmann, L.; Jehmlich, N.; Bernhardt, J.; Hecker, M.; Wollischeid, B.; Sun, Z.; *et al.* A peptide resource for the analysis of Staphylococcus aureus in host-pathogen interaction studies. *Proteomics* **2015**, *15*, 3648–3661. [CrossRef] [PubMed]

125. Hecker, M.; Becher, D.; Fuchs, S.; Engelmann, S. A proteomic view of cell physiology and virulence of *Staphylococcus aureus*. *Int. J. Med. Microbiol.* **2010**, *300*, 76–87. [CrossRef] [PubMed]

126. Singh, V.K.; Jayaswal, R.K.; Wilkinson, B.J. Cell wall-active antibiotic induced proteins of Staphylococcus aureus identified using a proteomic approach. *FEMS Microbiol. Lett.* **2001**, *199*, 79–84. [CrossRef]

127. Scherl, A.; Francois, P.; Charbonnier, Y.; Deshusses, J.M.; Koessler, T.; Huyghe, B.; Manto, M.; Stahl-Zeng, J.; Fischer, A.; Masselot, A.; *et al.* Exploring glycopeptide-resistance in Staphylococcus aureus: A combined proteomics and transcriptomics approach for the identification of resistance-related markers. *BMC Genom.* **2006**, *7*, 296. [CrossRef] [PubMed]

128. Fuchs, S.; Pane-Farre, J.; Kohler, C.; Hecker, M.; Engelmann, S. Anaerobic gene expression in *Staphylococcus aureus*. *J. Bacteriol.* **2007**, *189*, 4275–4289. [CrossRef] [PubMed]

129. Wolf, C.; Hochgrafe, F.; Kusch, H.; Albrecht, D.; Hecker, M.; Engelmann, S. Proteomic analysis of antioxidative strategies of *Staphylococcus aureus*: Diverse responses to different oxidants. *Proteomics* **2008**, *8*, 3139–3153. [CrossRef] [PubMed]

130. Hochgrafe, F.; Wolf, C.; Fuchs, S.; Liebeke, M.; Lalk, M.; Engelmann, S.; Hecker, M. Nitric oxide stress induces different responses but mediates comparable protein thiol protection in *Bacillus subtilis* and *Staphylococcus aureus*. *J. Bacteriol.* **2008**, *190*, 4997–5008. [CrossRef] [PubMed]

131. Resch, A.; Leicht, S.; Saric, M.; Pasztor, L.; Jakob, A.; Gotz, F.; Nordheim, A. Comparative proteome analysis of *Staphylococcus aureus* biofilm and planktonic cells and correlation with transcriptome profiling. *Proteomics* **2006**, *6*, 1867–1877. [CrossRef] [PubMed]

132. Fuchs, S.; Zühlke, D.; Pane-Farre, J.; Kusch, H.; Wolf, C.; Reiss, S.; Binh, T.N.; Albrecht, D.; Riedel, K.; Hecker, M.; *et al.* Aureolib—a proteome signature library: Towards an understanding of *Staphylococcus aureus* pathophysiology. *PLoS ONE* **2013**, *8*, e70669. [CrossRef] [PubMed]

133. Stentzel, S.; Sundaramoorthy, N.; Michalik, S.; Nordengrun, M.; Schulz, S.; Kolata, J.; Kloppot, P.; Engelmann, S.; Steil, L.; Hecker, M.; *et al.* Specific serum IgG at diagnosis of *Staphylococcus aureus* bloodstream invasion is correlated with disease progression. *J. Proteom.* **2015**, *128*, 1–7. [CrossRef] [PubMed]

134. Ziebandt, A.K.; Kusch, H.; Degner, M.; Jaglitz, S.; Sibbald, M.J.; Arends, J.P.; Chlebowicz, M.A.; Albrecht, D.; Pantucek, R.; Doe, J.; *et al.* Proteomics uncovers extreme heterogeneity in the *Staphylococcus aureus* exoproteome due to genomic plasticity and variant gene regulation. *Proteomics* **2010**, *10*, 1634–1644. [CrossRef] [PubMed]

135. Liew, Y.K.; Awang Hamat, R.; van Belkum, A.; Chong, P.P.; Neela, V. Comparative Exoproteomics and Host Inflammatory Response in *Staphylococcus aureus* Skin and Soft Tissue Infections, Bacteremia, and Subclinical Colonization. *Clin. Vaccine Immunol.* **2015**, *22*, 593–603. [CrossRef] [PubMed]

136. Bumann, D. Pathogen proteomes during infection: A basis for infection research and novel control strategies. *J. Proteom.* **2010**, *73*, 2267–2276. [CrossRef] [PubMed]

137. Schmidt, F.; Volker, U. Proteome analysis of host-pathogen interactions: Investigation of pathogen responses to the host cell environment. *Proteomics* **2011**, *11*, 3203–3211. [CrossRef] [PubMed]

138. Pförtner, H.; Burian, M.S.; Michalik, S.; Depke, M.; Hildebrandt, P.; Dhole, V.M.; Pane-Farre, J.; Hecker, M.; Schmidt, F.; Völker, U. Activation of the alternative sigma factor SigB of *Staphylococcus aureus* to the host cell environment. *Proteomics* **2011**, *11*, 177–187. [CrossRef] [PubMed]

139. Surmann, K.; Michalik, S.; Hildebrandt, P.; Gierok, P.; Depke, M.; Brinkmann, L.; Bernhardt, J.; Salazar, M.G.; Sun, Z.; Stetnberg, D.; *et al.* Comparative proteome analysis reveals conserved and specific adaptation patterns of *Staphylococcus aureus* after internalization by different types of human non-professional phagocytic host cells. *Front. Microbiol.* **2014**, *5*, 392. [CrossRef] [PubMed]

140. Steinhauser, C.; Heigl, U.; Tschikov, V.; Schwarz, J.; Gutsmann, T.; Seeger, K.; Brandenburg, J.; Fritsch, J.; Schroeder, J.; Wiesmueller, K.H.; *et al.* Lipid-labeling facilitates a novel magnetic isolation procedure to characterize pathogen-containing phagosomes. *Traffic* **2013**, *14*, 321–336. [CrossRef] [PubMed]
141. Mattow, J.; Siejak, F.; Hagens, K.; Becher, D.; Albrecht, D.; Krah, A.; Schmidt, F.; Jungblut, P.R.; Kaufmann, S.H.; Schaible, U.E. Proteins unique to intraphagosomally grown Mycobacterium tuberculosis. *Proteomics* 2006, 6, 2485–2494. [CrossRef] [PubMed]

142. Sturgill-Koszycki, S.; Schlesinger, P.H.; Chakraborty, P.; Haddix, P.L.; Collins, H.L.; Fok, A.K.; Allen, R.D.; Gluck, S.L.; Heuser, J.; Russell, D.G. Lack of acidification in Mycobacterium phagosomes produced by exclusion of the vesicular proton-ATPase. *Science* 1994, 263, 678–681. [CrossRef] [PubMed]

143. Luhrmann, A.; Haas, A. A method to purify bacteria-containing phagosomes from infected macrophages. *Methods Cell Sci. Off. J. Soc. Vitr. Biol.* 2000, 22, 329–341. [CrossRef]

144. Surmann, K.; Simon, M.; Hildebrandt, P.; Pförtner, H.; Michalik, S.; Stentzel, S.; Steil, L.; Dhople, V.M.; Bernhardt, J.; Schlüter, R.; et al. A proteomic perspective of the interplay of *Staphylococcus aureus* and human alveolar epithelial cells during infection. *J. Proteom.* 2015, 128, 203–217. [CrossRef] [PubMed]

145. Ventura, C.L.; Higdon, R.; Hohmann, L.; Martin, D.; Kolker, E.; Liggitt, H.D.; Skerrett, S.J.; Rubens, C.E. *Staphylococcus aureus* elicits marked alterations in the airway proteome during early pneumonia. *Infec. Immun.* 2008, 76, 5862–5872. [CrossRef] [PubMed]

146. van Schaik, B.; Klarenbeek, P.; Doorenspleet, M.; van Kampen, A.; Moody, D.B.; de Vries, N.; Van Rhijn, I. Discovery of invariant T-cells by next-generation sequencing of the human TCR alpha-chain repertoire. *J. Immunol.* 2014, 193, 5338–5344. [CrossRef] [PubMed]

147. Wine, Y.; Horton, A.P.; Ippolito, G.C.; Georgiou, G. Serology in the 21st century: The molecular-level analysis of the serum antibody repertoire. *PLoS ONE* 2015, 10, e013494. [CrossRef] [PubMed]

148. Lavinder, J.J.; Horton, A.P.; Georgiou, G.; Ippolito, G.C. Next-generation sequencing and protein mass spectrometry for the comprehensive analysis of human cellular and serum antibody repertoires. *Curr. Opin. Chem. Biol.* 2015, 24, 112–120. [CrossRef] [PubMed]

149. Al-Yahya, S.; Mahmoud, L.; Al-Zoghaibi, F.; Al-Tuhami, A.; Amer, H.; Almajhdi, F.N.; Polyak, S.J.; Khabar, K.S. Unexpectedly high T-cell memory response to *Staphylococcus aureus* in humans. *PLoS ONE* 2014, 9, e97581. [CrossRef] [PubMed]

150. Derycke, L.; Eyerich, S.; Van Crombruggen, K.; Perez-Novó, C.; Holtappels, G.; Deruyck, N.; Gevaert, P.; Bachert, C. Mixed T helper cell signatures in chronic rhinosinusitis with and without polyps. *PLoS ONE* 2015, 10, e88130. [CrossRef] [PubMed]

151. Kolata, J.; Kühlbandner, I.; Link, C.; Normann, N.; Weidenmaier, C.; Bröker, B. The fall of a dogma? Unexpectedly high T-cell memory response to *Staphylococcus aureus* bacteremia in humans. *J. Infect. Dis.* 2015, 212, 830–836. [CrossRef] [PubMed]

152. Ricciardi-Castagnoli, P.; Granucci, F. Opinion: Interpretation of the complexity of innate immune responses by functional genomics. *Nat. Rev. Immunol.* 2002, 2, 881–889. [CrossRef] [PubMed]

153. Hyatt, G.; Melamed, R.; Park, R.; Seguritan, R.; Laplace, C.; Poirot, L.; Zucchelli, S.; Obst, R.; Matos, M.; Venanzi, E.; et al. Gene expression microarrays: Glimpses of the immunological genome. *Nat. Immunol.* 2006, 7, 686–691. [CrossRef] [PubMed]

154. Georgiou, G.; Ippolito, G.C.; Beausang, J.; Busse, C.E.; Wardemann, H.; Quake, S.R. The promise and challenge of high-throughput sequencing of the antibody repertoire. *Nat. Biotechnol.* 2014, 32, 158–168. [CrossRef] [PubMed]

155. Doolan, D.L. Plasmodium immunomics. *Int. J. Parasitol.* 2011, 41, 3–20. [CrossRef] [PubMed]

156. Bröker, B.M.; van Belkum, A. *Staphylococcus aureus* immune proteomics. *Proteomics* 2011, 11, 3221–3231. [CrossRef] [PubMed]

157. Kolata, J.; Bode, L.G.; Holtfreter, S.; Steil, L.; Kusch, H.; Holtfreter, B.; Albrecht, D.; Hecker, M.; Engelmann, S.; van Belkum, A.; et al. Distinctive patterns in the human antibody response to *Staphylococcus aureus* bacteremia in carriers and non-carriers. *Proteomics* 2011, 11, 3914–3927. [CrossRef] [PubMed]

158. Tjalsma, H.; Schaeps, R.M.; Swinkels, D.W. Immunoproteomics: From biomarker discovery to diagnostic applications. *Proteom. Clin. Appl.* 2008, 2, 167–180. [CrossRef] [PubMed]

159. Etz, H.; Minh, D.; Henics, T.; Dryla, A.; Winkler, B.; Triska, C.; Boyd, A.; Söllner, J.; Schmidt, W.; von Ahlsen, U.; et al. Identification of in vivo expressed vaccine candidate antigens from *Staphylococcus aureus*. *Proc. Natl. Acad. Sci. USA.* 2002, 99, 6575–6578. [CrossRef] [PubMed]
161. Verkai, N.; Brouwer, E.; Hooijkaas, H.; van Belkum, A.; van Wamel, W. Comparison of carboxylated and Penta-His microspheres for semi-quantitative measurement of antibody responses to His-tagged proteins. J. Immunol. Methods 2008, 335, 121–125. [CrossRef] [PubMed]

162. Verkai, N.J.; Boelens, H.A.; de Vogel, C.P.; Tavakol, M.; Bode, L.G.; Verbrugh, H.A.; van Belkum, A.; van Wamel, W.J. Heterogeneity of the humoral immune response following Staphylococcus aureus bacteremia. Eur. J. Clin. Microbiol. Infect. Dis. 2010, 29, 509–518. [CrossRef] [PubMed]

163. Kloppot, P.; Selle, M.; Kohler, C.; Stentzel, S.; Fuchs, S.; Liebscher, V.; Muller, E.; Kale, D.; Ohlsen, K.; Broker, B.M.; et al. Microarray-based identification of human antibodies against Staphylococcus aureus antigens. Proteomics Clin. Appl. 2015, 9, 1003–1011. [CrossRef] [PubMed]

164. Colque-Navarro, P.; Palma, M.; Soderquist, B.; Flock, J.I.; Mollby, R. Antibody responses in patients with staphylococcal septicaemia against two Staphylococcus aureus fibrinogen binding proteins: Clumping factor and an extracellular fibrinogen binding protein. Clin. Diagn. Lab. Immunol. 2000, 7, 14–20. [CrossRef] [PubMed]

165. Verkai, N.J.; Lebon, A.; de Vogel, C.P.; Hooijkaas, H.; Verbrugh, H.A.; Jaddoe, V.W.; Hofman, A.; Moll, H.A.; van Belkum, A.; van Wamel, W.J. Induction of antibodies by Staphylococcus aureus nasal colonization in young children. Clin. Microbiol. Infect. 2009, 16, 1312–1317. [CrossRef] [PubMed]

166. Colque-Navarro, P.; Jacobsson, G.; Andersson, R.; Flock, J.I.; Mollby, R. Levels of antibody against 11 Staphylococcus aureus antigens in a healthy population. Clin. Vaccine Immunol. 2010, 17, 1117–1123. [CrossRef] [PubMed]

167. Holtfreter, S.; Roschack, K.; Eichler, P.; Eske, K.; Holtfreter, B.; Kohler, C.; Engelmann, S.; Hecker, M.; Greinacher, A.; Bröker, B.M. Staphylococcus aureus carriers neutralize superantigens by antibodies specific for their colonizing strain: A potential explanation for their improved prognosis in severe sepsis. J. Infect. Dis. 2006, 193, 1275–1278. [CrossRef] [PubMed]

168. Ghasemzadeh-Moghaddam, H.; Neela, V.; van Wamel, W.; Hamat, R.A.; Shamsuddin, M.N.; Hussin, N.S.; Aziz, M.N.; Haspani, M.S.; Johar, A.; Thevarajah, S.; et al. Nasal carriers are more likely to acquire exogenous Staphylococcus aureus strains than non-carriers. Clin. Microbiol. Infect. 2015, 21, 998.e1–998.e7. [CrossRef] [PubMed]

169. Van der Kooi-Pol, M.M.; de Vogel, C.P.; Westerhout-Pluister, G.N.; Veenstra-Kyuchukova, Y.K.; Duipmans, J.C.; Glasner, C.; Buist, G.; Elsinga, G.S.; Westra, H.; Bonarius, H.P.; et al. High anti-staphylococcal antibody titers in patients with epidermolysis bullosa relate to long-term colonization with alternating types of Staphylococcus aureus. J. Investig. Dermatol. 2013, 133, 847–850. [CrossRef] [PubMed]

170. Van der Kooi-Pol, M.M.; Duipmans, J.C.; Jonkman, M.F.; van Dijl, J.M. Host-pathogen interactions in epidermolysis bullosa patients colonized with Staphylococcus aureus. Int. J. Med. Microbiol. 2014, 304, 195–203. [CrossRef] [PubMed]

171. O’hea, J. Helper T-cell differentiation and plasticity. In Fundamental Immunology, 7th ed.; Paul, W.E., Ed.; Lippincott Williams & Wilkins: Philadelphia, PA, USA, 2013; pp. 708–717.

172. McHeyzer-Williams, M.; Okitsu, S.; Wang, N.; McHeyzer-Williams, L. Molecular programming of B-cell memory. Nat. Rev. Immunol. 2012, 12, 24–34. [CrossRef] [PubMed]

173. Montgomery, C.P.; David, M.Z.; Daum, R.S. Host factors that contribute to recurrent staphylococcal skin infection. Curr. Opin. Infect. Dis. 2015, 28, 253–258. [CrossRef] [PubMed]

174. Murphy, A.G.; O’Keeffe, K.M.; Lalor, S.J.; Maher, B.M.; Mills, K.H.; McLoughlin, R.M. Staphylococcus aureus infection of mice expands a population of memory gammadelta T-cells that are protective against subsequent infection. J. Immunol. 2014, 192, 3697–3708. [CrossRef] [PubMed]

175. Cho, J.S.; Pietras, E.M.; Garcia, N.C.; Ramos, R.I.; Farzam, D.M.; Monroe, H.R.; Magorien, J.E.; Blauvelt, A.; Kolls, J.K.; Cheung, A.L.; et al. IL-17 is essential for host defense against cutaneous Staphylococcus aureus infection in mice. J. Clin. Invest. 2010, 120, 1762–1773. [CrossRef] [PubMed]

176. Ishigame, H.; Kakuta, S.; Nagai, T.; Kadoki, M.; Nambu, A.; Komiyama, Y.; Fujikado, N.; Tanahashi, Y.; Akitsu, A.; Kotaki, H.; et al. Differential roles of interleukin-17A and -17F in host defense against mucocutaneous bacterial infection and allergic responses. Immunity 2009, 30, 108–119. [CrossRef] [PubMed]

177. Kudva, A.; Scheller, E.V.; Robinson, K.M.; Crowe, C.R.; Choi, S.M.; Slight, S.R.; Khader, S.A.; Dubin, P.J.; Enelow, R.I.; Kolls, J.K.; et al. Influenza A inhibits Th17-mediated host defense against bacterial pneumonia in mice. J. Immunol. 2011, 186, 1666–1674. [CrossRef] [PubMed]
178. Lin, L.; Ibrahim, A.S.; Xu, X.; Farber, J.M.; Avanesian, V.; Baquir, B.; Fu, Y.; French, S.W.; Edwards, J.E., Jr.; Spellberg, B. Th1-Th17 cells mediate protective adaptive immunity against Staphylococcus aureus and Candida albicans infection in mice. *PLoS Pathog.* 2009, 5, e1000703. [CrossRef] [PubMed]

179. Archer, N.K.; Harro, J.M.; Shirliff, M.E. Clearance of Staphylococcus aureus nasal carriage is T-cell dependent and mediated through interleukin-17A expression and neutrophil influx. *Infect. Immun.* 2013, 81, 2070–2075. [CrossRef] [PubMed]

180. Fraunholz, M.; Sinha, B. Intracellular Staphylococcus aureus: Live-in and let die. *Front. Cell. Infect. Microbiol.* 2012, 2, 43. [CrossRef] [PubMed]

181. Grumann, D.; Nubel, U.; Broker, B.M. Staphylococcus aureus toxins— their functions and genetics. *BMC Struct. Biol.* 2014, 178, 199–230. [CrossRef] [PubMed]

182. Bagnoli, F.; Baudner, B.; Mishra, R.P.; Bartolini, E.; Fiaschi, L.; Mariotti, P.; Nardi-Dei, V.; Boucher, P.; Rappuoli, R. Reverse vaccinology, a genome-based approach to vaccine development. *J. Bacteriol.* 2013, 195, 2688–2691. [CrossRef] [PubMed]

183. Ammons, M.C.; Tripet, B.P.; Carlson, R.P.; Kirker, K.R.; Gross, M.A.; Stanisich, J.J.; Copie, V. Quantitative NMR metabolite profiling of methicillin-resistant and methicillin-susceptible Staphylococcus aureus discriminates between biofilm and planktonic phenotypes. *J. Proteome Res.* 2014, 13, 2973–2985. [CrossRef] [PubMed]

184. Lochner, M.; Berod, L.; Sparwasser, T. Fatty acid metabolism in the regulation of T-cell function. *J. Exp. Med.* 2013, 2688–2691. [CrossRef] [PubMed]

185. Berube, B.J.; Bubeck Wardenburg, J. Staphylococcus aureus alpha-toxin: Nearly a century of intrigue. *PLoS Pathog.* 2012, 8, e1002550. [CrossRef] [PubMed]

186. Liebeke, M.; Lalk, M. Staphylococcus aureus metabolic response to changing environmental conditions— A metabolomics perspective. *Int. J. Med. Microbiol.* 2014, 304, 222–229. [CrossRef] [PubMed]

187. Alonzo, F., III; Torres, V.J. The bicomponent pore-forming leucocidins of Staphylococcus aureus. *Microbiol. Mol. Biol. Rev.* 2014, 78, 81–91. [CrossRef] [PubMed]

188. Dorries, K.; Schlueter, R.; Lalk, M. Impact of antibiotics with various target sites on the metabolome of Staphylococcus aureus reveals changes in the metabolic landscape. *Front. Cell. Infect. Microbiol.* 2013, 3, 545–566. [CrossRef] [PubMed]

189. Villa, I.; Karmali, M.A.; Khodadoust, A.; Tummy, J.; Zaidi, S.; Wu, J.; Desai, N.; Hafiz, M.; Gontareva, T.; Wang, B.; et al. Identification of candidate vaccine antigens. *Immunome Res.* 2010, 6 (Suppl. 2), S1. [CrossRef] [PubMed]

190. Bruno, L.; Cortese, M.; Rappuoli, R.; Merola, M. Lessons from Reverse Vaccinology for viral vaccine design. *Curr. Opin. Virol.* 2015, 11, 89–97. [CrossRef] [PubMed]

191. Flower, D.R.; Macdonald, I.K.; Ramakrishnan, K.; Davies, M.N.; Doytchinova, I.A. Computer aided selection of candidate vaccine antigens. *Immunome Res.* 2010, 6 (Suppl. 2), S1. [CrossRef] [PubMed]

192. Hoerr, V.; Zbytnuik, L.; Leger, C.; Tam, P.P.; Kubes, P.; Vogel, H.J. Gram-negative and Gram-positive bacterial infections give rise to a different metabolic response in a mouse model. *J. Proteome Res.* 2012, 11, 3231–3245. [CrossRef] [PubMed]

193. Slupsky, C.M.; Chey, J.; Chao, D.V.; Fu, H.; Rankin, K.N.; Marrie, T.J.; Lacy, P. Streptococcus pneumoniae and Staphylococcus aureus pneumonia induce distinct metabolic responses. *J. Proteome Res.* 2009, 8, 3029–3036. [CrossRef] [PubMed]

194. Bruno, L.; Cortese, M.; Rappuoli, R.; Merola, M. Lessons from Reverse Vaccinology for viral vaccine design. *Curr. Opin. Virol.* 2015, 11, 89–97. [CrossRef] [PubMed]

195. Flower, D.R.; Macdonald, I.K.; Ramakrishnan, K.; Davies, M.N.; Doytchinova, I.A. Computer aided selection of candidate vaccine antigens. *Immunome Res.* 2010, 6 (Suppl. 2), S1. [CrossRef] [PubMed]

196. Bruno, L.; Cortese, M.; Rappuoli, R.; Merola, M. Lessons from Reverse Vaccinology for viral vaccine design. *Curr. Opin. Virol.* 2015, 11, 89–97. [CrossRef] [PubMed]
200. Sela-Culang, I.; Ofran, Y.; Peters, B. Antibody specific epitope prediction-emergence of a new paradigm. *Curr. Opin. Virol.* 2015, 11, 98–102. [CrossRef] [PubMed]

201. Vita, R.; Overton, J.A.; Greenbaum, J.A.; Ponomarenko, J.; Clark, J.D.; Cantrell, J.R.; Wheeler, D.K.; Gabbard, J.L.; Hix, D.; Sette, A.; *et al.* The immune epitope database (IEDB) 3.0. *Nucleic Acids Res.* 2015, 43, D405–D412. [CrossRef] [PubMed]

202. Yao, B.; Zheng, D.; Liang, S.; Zhang, C. Conformational B-cell epitope prediction on antigen protein structures: A review of current algorithms and comparison with common binding site prediction methods. *PLoS ONE* 2013, 8, e62249. [CrossRef] [PubMed]

203. Doytchinova, I.A.; Flower, D.R. Identifying candidate subunit vaccines using an alignment-independent method based on principal amino acid properties. *Vaccine* 2007, 25, 856–866. [CrossRef] [PubMed]

204. Altindis, E.; Cozzi, R.; di Palo, B.; Necchi, F.; Mishra, R.P.; Fontana, M.R.; Soriani, M.; Bagnoli, F.; Maione, D.; Grandi, G.; *et al.* Protectome analysis: A new selective bioinformatics tool for bacterial vaccine candidate discovery. *Mol. Cell. Proteom.* 2015, 14, 418–429. [CrossRef] [PubMed]

205. Oprea, M.; Antohe, F. Reverse-vaccinology strategy for designing T-cell epitope candidates for *Staphylococcus aureus* endocarditis vaccine. *Biol. J. Int. Assoc. Biol. Stand.* 2013, 41, 148–153. [CrossRef] [PubMed]

206. Glowalla, E.; Tosetti, B.; Krönke, M.; Krut, O. Proteomics-based identification of anchorless cell wall proteins as vaccine candidates against *Staphylococcus aureus*. *Infect. Immun.* 2009, 77, 2719–2729. [CrossRef] [PubMed]

207. Vytyvtyska, O.; Nagy, E.; Bluggel, M.; Meyer, H.; Kurzbauer, R.; Huber, L.; Klade, C. Identification of vaccine candidate antigens of *Staphylococcus aureus* by serological proteome analysis. *Proteomics* 2002, 2, 580–590. [CrossRef] [PubMed]

208. Glasner, C.; van Timmeren, M.M.; Stobernack, T.; Omansen, T.F.; Raangs, E.C.; Rossen, J.W.; de Goffau, M.C.; Arends, J.P.; Kampinga, G.A.; Koedijk, D.G.; *et al.* Low anti-staphylococcal IgG responses in granulomatosis with polyangiitis patients despite long-term *Staphylococcus aureus* exposure. *Sci. Rep.* 2015, 5, 8188. [CrossRef] [PubMed]

209. Swierstra, J.; Debets, S.; de Vogel, C.; Lemmens-den Toom, N.; Verkaik, N.; Ramdani-Bouguessa, N.; Jonkman, M.F.; van Dijl, J.M.; Fahal, A.; van Belkum, A.; *et al.* IgG4 subclass-specific responses to *Staphylococcus aureus* antigens shed new light on host-pathogen interaction. *Infect. Immun.* 2015, 83, 492–501. [CrossRef] [PubMed]

210. Den Reijer, P.M.; Lemmens-den Toom, N.; Kant, S.; Snijders, S.V.; Boelens, H.; Tavakol, M.; Verkaik, N.J.; van Belkum, A.; Verbrugh, H.A.; van Wamel, W.J. Characterization of the humoral immune response during *Staphylococcus aureus* bacteremia and global gene expression by *Staphylococcus aureus* in human blood. *PLoS ONE* 2013, 8, e53391.

© 2016 by the authors; licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons by Attribution (CC-BY) license (http://creativecommons.org/licenses/by/4.0/).