Pathogenesis of *Staphylococcus aureus* and Proteomic Strategies for the Identification of Drug Targets

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Abstract

Staphylococcus aureus is a leading pathogen responsible for mild to severe invasive infections in humans. Especially, methicillin-resistant *Staphylococcus aureus* (MRSA) is prevalent in hospital settings and biomaterial-associated infections. In addition, MRSA is listed as high-priority pathogen in WHO priority pathogen list and occupied the serious threat level in CDC's drug-resistant bacteria report. Persistent *S. aureus* infections are often associated with biofilm formation and resistant to conventional antimicrobial therapy. Inhibiting the surface adherence and virulence of the bacterium is the current alternative approach without affecting growth to reduce the possibility of resistance development. Though numerous antibiofilm agents have been identified, their mode of action remains unclear. Proteomics is the powerful approach to delineate the drug targets of bioactive molecules. Bottom-up strategy-based comparative proteomics is extensively used in the field of disease diagnosis and therapy. Molecular targets of antibiotics and antibiofilm agents active against *S. aureus* have been unveiled using various proteomic approaches and lead to development of drug discovery as well.

Keywords

Staphylococcus aureus · Pathogenesis · Biofilm formation · Proteomics · Drug target discovery

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18.1 Introduction

S. aureus is a dangerous bacterium capable of causing deadly invasive infections in humans in addition to mild superficial skin infections. With a plethora of virulence determinants, *S. aureus* is able to adhere to biotic and abiotic surfaces and survive even under adverse host conditions. Especially, *S. aureus* is the predominant cause of biomaterial-associated infections as this pathogen prefers to adhere to the foreign materials inside the body and mostly leads to failure of implanted devices. The major complexity associated with persistent implant infections is formation of biofilm which endows the bacterial cells with the resistance nature [1]. Due to resistance to majority of commonly available antibiotics, the medical community is left with a very few options to treat *S. aureus* infections. Instead of killing the bacteria with antibiotics, inhibition of biofilm formation with antibiofilm agents seems to be a good alternative strategy to fight bacterial infections in the recent times [2]. Apart from finding the potential antibiofilm agents, understanding their mechanism of action is also equally important. Thus, novel drugs with effective mode of action can be synthesized. In addition, toxicity of drug molecules can be ruled out when precise mode of action is known. Proteomic approach gives an in-depth understanding of expression of virulence determinants in *S. aureus* and uncovers the complexity of the virulence machineries involved in pathogenicity. The proteomic approach utilizes various techniques which can be generally classified into two categories, namely, gel-based and gel-free. Various proteomic strategies developed with advancements shed more light on elucidation of drug targets in *S. aureus* antibiotic resistance and contribute to the progression of antistaphylococcal therapy and drug discovery [3]. This chapter elaborates the virulence attributes of *S. aureus* and emphasizes the efficacy of proteomics in drug target identification.

18.2 *S. aureus* Infections in Humans

S. aureus is a human commensal bacterium mostly present in the skin and mucosae. Though various body sites can be colonized by *S. aureus*, the anterior nares of the nose is the frequent and predominant carriage site of *S. aureus* in humans [4]. *S. aureus* colonizes anterior nares of 20–80% of the human population at any stage of life, and 30% of human population is constantly colonized with *S. aureus* [5]. The commensal *S. aureus* turns pathogenic when the individual becomes immune compromised and weak [6]. Various infections caused by *S. aureus* in humans are depicted in Fig. 18.1. *S. aureus* majorly causes skin and soft tissue infections such as abscesses, sores, impetigo, boils, lesions, cellulitis, and folliculitis. Apart from these minor infections, *S. aureus* can also cause life-threatening invasive infections such as bacteremia, pneumonia, osteomyelitis, septic arthritis, otitis media, endocarditis, meningitis, and indwelling device-related infections [7, 8]. In the recent decades, the epidemiology of *S. aureus* gained more global attention because of the high incidence of *S. aureus* in healthcare-associated infections [9].

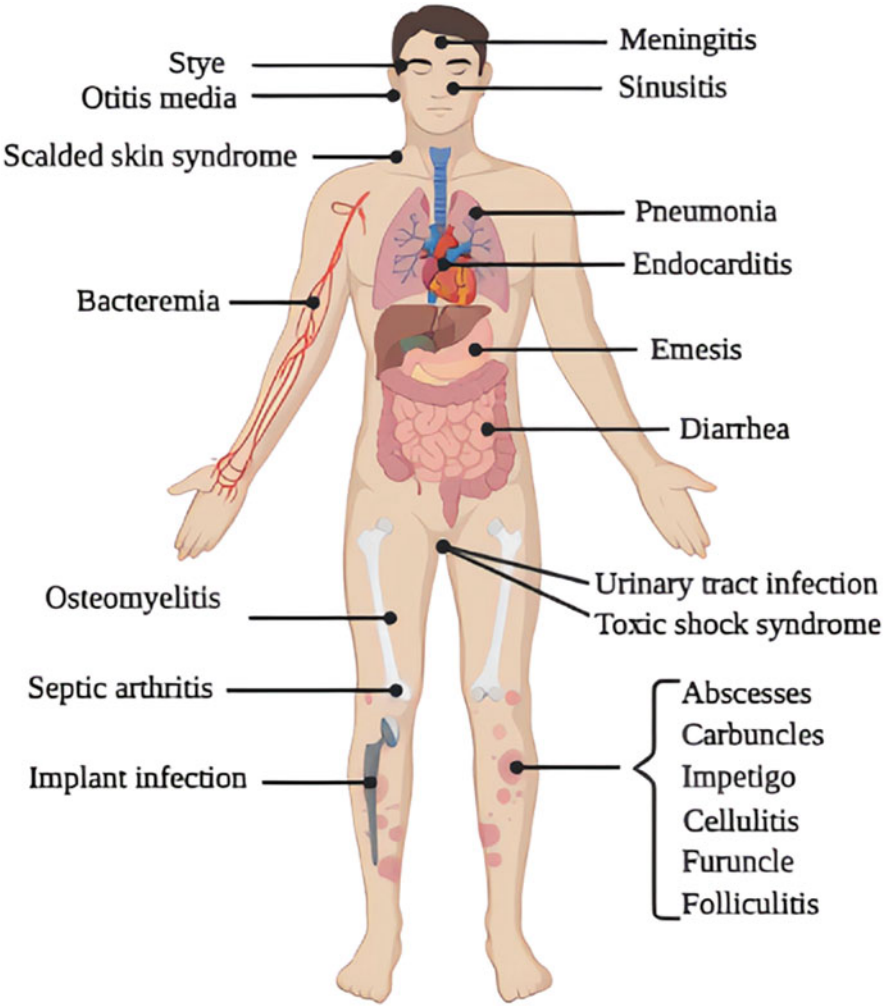

Fig. 18.1 Graphical representation of moderate to severe infections caused by *S. aureus* in humans (Created in BioRender.com)

Most notably, *S. aureus* is the predominant pathogen isolated from a variety of implantable medical devices [10]. Extensive usage of implants poses serious problems to the patients by damaging epithelial or mucosal barriers and thereby supports invasion of microorganisms which serve as reservoir of microbial infections [11]. It has been reported that more than 45% of nosocomial infections is caused by means of implanted medical devices. Further research on investigation of microbial community associated with implant infection has revealed *S. aureus* as most dangerous bacterium which can colonize the implanted surface in an irreversible manner [1]. Specifically, *S. aureus* has been frequently encountered in patients

with infective endocarditis and prosthetic device-associated infections [12]. Mortality and morbidity rate of *S. aureus* infections is steadily increasing as prevalence of *S. aureus* became ineradicable [13]. In 2017, the World Health Organization (WHO) released the global priority list of antibiotic-resistant bacteria in which MRSA occupied the high priority [14]. In addition, MRSA was listed as the serious threats in the antibiotic resistance threat report published by the Centers for Disease Control and Prevention (CDC) in 2019 [15].

18.3 Antibiotic Resistance in *S. aureus*: A Global Threat

Interestingly, the world's first antibiotic was discovered from the contaminated plate of *S. aureus*. The story of antibiotics started in the year 1928 when Sir Alexander Fleming, a Scottish physician and microbiologist, accidentally discovered penicillin from the fungus *Penicillium notatum* which contaminated the *S. aureus* plate left opened in his laboratory in Paddington, London [16, 17]. After 12 years from discovery, the pure form of penicillin was made clinically available in the year 1941 which saved the life of numerous soldiers with bacterial pneumonia and meningitis during the Second World War. Due to the ability to cure various bacterial infections, penicillin earned the repute of being called as a "wonder drug" or a "miracle drug." From then, different classes of novel antibiotics were produced, and commercial availability of many antibiotics happened in the period of 1950–1970 which is referred to as "golden era of antibiotics" [18, 19].

Shockingly, a report on penicillin-resistant *S. aureus* was published in 1940 which was even before the first clinical use of penicillin [20]. Fleming's comment on resistance in his Nobel lecture in the year 1945 was even more surprising. He mentioned that "But I would like to sound one note of warning. Penicillin is to all intents and purposes non-poisonous so there is no need to worry about giving an overdose and poisoning the patient. There may be a danger, though, in under dosage. It is not difficult to make microbes resistant to penicillin in the laboratory by exposing them to concentrations not sufficient to kill them and the same thing has occasionally happened in the body" [21].

Later in 1959, Celbenin (with trade name of methicillin), a penicillinase-resistant penicillin, was launched to fight against penicillin-resistant *S. aureus*. Methicillin was considered to be the end of staphylococcal resistance. However, within a very short span of time, methicillin-resistant *S. aureus* (MRSA) was identified by M. Patricia Jevons from Staphylococcus Reference Laboratory, London, in 1960. *mecA* gene encoding penicillin-binding protein PBP 2A is responsible for methicillin resistance. Over the time, *mecA* gene got spread worldwide and 60–70% of *S. aureus* strains are reported to be methicillin resistant [22]. Vancomycin was approved by the Food and Drug Administration (FDA) in the year 1958 with an aim to treat bacterial strains resistant to methicillin though it was identified earlier in 1953. The first clinical strain of *S. aureus* with reduced susceptibility to vancomycin isolated in Japan in 1996 was named as vancomycin-intermediate *S. aureus* (VISA) and clinical isolate of *S. aureus* with resistance to vancomycin detected in the United States in

2002 was named as vancomycin-resistant *S. aureus* (VRSA) [23, 24]. After the global emergence of VISA and VRSA, the new antibiotic linezolid was approved for clinical use in 2000. Unsurprisingly, linezolid-resistant staphylococci were reported shortly in 2001 [25].

The historical events manifest the ability of *S. aureus* to acquire resistance to a variety of drugs in a short period of time, whereas the discovery of every antibiotic taken a long span of time and immense efforts. From 1970, numerous MRSA strains with multiple drug resistance (MDR) were identified and made MRSA superbug worldwide. Global spread of drug resistance diminished the value of antibiotics in the treatment of bacterial infections [26]. Hence, the MRSA infections are hard to cure with limited efficacy of antibiotics and evolved as serious clinical issue which challenges the clinicians as well as researchers. Evolution of MDR and therapeutic failure of antibiotics led to the post-antibiotic era to overcome severe bacterial infections [27].

18.4 Pathogenesis of *S. aureus*

18.4.1 Repertoire of Virulence Factors

S. aureus is capable of producing plenty of structural and secreted virulence factors involved in pathogenesis. *S. aureus* produces numerous surface proteins, called "microbial surface components recognizing adhesive matrix molecules" (MSCRAMMs), which mediate adherence of bacterial cells to host tissues. MSCRAMMs specifically bind to the major components of host tissue such as collagen, fibronectin, and fibrinogen. MSCRAMMs play a critical role in the initiation of endovascular infections and biomaterial-associated infections. Once *S. aureus* colonized on host tissues or prosthetic surfaces, it is able to survive and persist in several ways. *S. aureus* has various virulence traits to evade the host immune system during establishment of an infection [28]. The major virulence factors produced by *S. aureus* and their role in pathogenesis are presented in Table 18.1.

During the progression of infection, *S. aureus* secrets various enzymes such as elastases, lipases, and proteases to invade and destroy host tissues. These secretary virulence factors help metastasize to the new sites to disseminate the infection. The structural virulence components such as peptidoglycan and lipoteichoic acid play a role in activation of the host immune system and coagulation pathways to produce septic shock. Apart from septic shock, *S. aureus* also produces superantigens that cause toxic shock syndrome and food poisoning [49, 50]. Expression of virulence factors is metabolically expensive and occurs in a highly controlled manner. MSCRAMM adhesion proteins are generally expressed during logarithmic growth phase to facilitate initial adhesion, whereas secretary enzymes and toxins are produced during the stationary phase to progress and disseminate the infection [51].

Table 18.1 Major virulence factors of *S. aureus*

Virulence factor	Biological function	Reference
Adhesins		
Teichoic acid	Highly charged cell wall polymers, play a key role in the first step of biofilm formation	[29]
Intracellular adhesion (Ica)	Synthesize polysaccharide namely poly N-acetyl glucosamine (PIA/PNAG) involved in biofilm formation	[30]
Staphylococcal protein A (Spa)	A surface-anchored structural protein contributes to colonization and immune evasion. Blocks opsonophagocytosis through nonspecific interaction with Fc portion of the immunoglobulin G (IgG)	[31]
Fibronectin-binding proteins (FnbA and FnbB)	Contribute to tissue colonization in various pathological conditions and indwelling medical device-related infections	[32]
Elastin-binding protein (Ebp)	An integral membrane protein mediates adherence of bacterial cells to specific components of extracellular matrix	[33]
Collagen adhesion (Cna)	Facilitates binding of *S. aureus* to bone matrix	[34]
Clumping factors (ClfA and ClfB)	A cell wall-anchored protein promotes bacterial adhesion to the blood plasma protein fibrinogen and colonization on protein-coated biomaterials	[35]
Autolysin A (AtlA)	A cell surface-associated peptidoglycan hydrolase promotes attachment to polystyrene surfaces and play important role in biofilm development	[36]
Enzymes		
Metalloprotease; aureolysin (Aur)	Belongs to the family of thermolysins, have a role in staphylococcal immune escape by cleaving complement proteins	[37]
Staphopain proteases (SspA, SspB and SspC)	Important immunomodulatory proteins that inhibit phagocytosis and neutrophil recruitment and damage the epithelium and underlying connective tissue. Also involved in biofilm dispersal	[38]
Lipase (Geh/Lip2)	Interfere with the host granulocyte function, and increase survival of the bacteria against the host defense by inactivating bactericidal lipids	[39]
Nuclease (Nuc)	Required for the evasion of neutrophil extracellular traps (NETs) and has a role in the inhibition of biofilm formation	[40]
Catalase (KatA)	An enzyme implicated in oxidative stress resistance and protects intraphagocytic bacteria by destroying hydrogen peroxide produced by the phagocyte	[41]
Hyaluronidase (HysA)	Promotes tissue penetration and disease progression	[42]
Coagulase (Coa)	Activates prothrombin, thereby converting fibrinogen to fibrin and promoting clotting of plasma or blood. Also responsible for abscess formation and persistence in host tissues	[43]

(continued)

Table 18.1 (continued)

Virulence factor	Biological function	Reference
Toxins		
Phenol-soluble modulins (PSMs)	Efficiently lyse white and red blood cells and contribute to the structuring of biofilms and the dissemination of biofilm-associated infections	[44]
Hemolysins (Hld and Hla)	Induces lysis of red blood cells and play an important role in various diseases such as pneumonia, sepsis, septic arthritis, brain abscess, and corneal infections	[45]
Staphylococcal enterotoxins (Sea and Seb)	Enter the bloodstream and circulate through the body, thus allowing the interaction with antigen-presenting cells and T cells that leads to superantigen activity and causes classic food poisoning, nausea, vomiting, and diarrhea without fever	[46]
Panton-Valentine leukocidin (PVL)	A potent pore-forming cytotoxin causes tissue necrosis and selectively disrupts leukocyte membranes, thus leading to enhanced virulence	[47]
Toxic shock syndrome toxin-1 (TSST-1)	A prototype-secreted superantigen binds to class II MHC molecules on antigen-presenting cells and stimulate large populations of T cells leading to an acute toxic shock	[48]

18.4.2 Biofilm Formation

What makes the tiny *S. aureus* to acquire the ability to infect giant humans and even to cause death is just the group behavior. Discovery of bacterial communication otherwise known as quorum sensing not only awakened the global researchers to focus on virulence nature of microorganisms and also unearthed the multi-cellular behavior of microorganisms [52]. Bacteria achieved the eukaryotic lifestyle by adherence based community living in the form of biofilm. Bacterial biofilms are the sessile and highly structured microbial communities which are encased within the self-produced matrix of extracellular polymeric substances (EPS). Biofilm mode of lifestyle enables the bacterium to adhere onto both biotic and abiotic surfaces [53].

S. aureus is a well-known biofilm-producing bacterium and plays a crucial role in hospital-associated infections by forming biofilm on medical devices. Biofilm formation in *S. aureus* is a multistep process. Initial step of biofilm formation is adherence to either biotic or abiotic surfaces using adhesin proteins which is followed by the proliferation of cells to form microcolonies, and then the secretion of EPS induces more cells to form a three-dimensional biofilm. EPS is a hydrated three-dimensional matrix comprising polysaccharides along with molecules such as eDNA, eProteins, and lipids. Once mature biofilm is formed, it becomes a stable microbial community against adverse environmental conditions. Dispersal of biofilm is mediated by the production of matrix-degrading enzymes such as nucleases and proteases [54].

Formation of biofilm provides the adherent stay for the bacteria, thereby making them more virulent than planktonic cells in numerous ways such as resistance to host immune response, altered growth rate, metabolically inactive persister cell

formation, synchronized virulence gene expression, and horizontal gene transfer [55, 56]. Hence, antibiotics are unable to penetrate the slimy EPS matrix of biofilm. In addition, gene encoding antibiotic resistance is highly transferred within biofilm cells, and hence antibiotic-degrading enzymes are overexpressed in biofilm cells, thereby making the whole microbial community antibiotic resistant.

18.4.3 Regulatory Mechanisms of Biofilm Formation and Virulence

The formation of biofilm in *S. aureus* is well organized and tightly controlled as well. A complex network of regulatory molecules controls the expression of biofilm components either positively or negatively [57]. Bacterial cells secrete as well as detect the signaling molecules also called as autoinducing peptides (AIP) which elicit the cascade of biological processes inside a cell. This kind of bacterial communication is called quorum sensing which regulates the expression of virulence traits. In *S. aureus*, quorum sensing is mediated by accessory gene regulatory (*agr*) system which consists of *agrBDCA* operon. AIP-mediated *agr* system regulates the production of an array of structural and secreted virulence factors in *S. aureus*. Agr is a two-component regulatory system controlled by *agr* operon with four genes *agrBDCA* in which *agrD* codes for AIP which is further processed and transported by *agrB* and extracellular AIP is recognized by the receptor protein *agrC* which phosphorylates the cytoplasmic partner *agrA* which further induces the expression of regulatory RNA known as RNAIII as well as induces the expression of *agrBDCA* as feedforward induction. RNAIII inhibits the production of adhesion proteins which are involved in colonization whereas induces the production of matrix-degrading enzymes which are involved in dissemination. Thus, *agr* system acts as the switch between biofilm and planktonic state of bacterial growth depending on the cell density [58, 59].

Apart from *agr* system, various regulators are involved in governing biofilm formation. A major regulatory molecule appears to play a key role is staphylococcal accessory regulator A (*sarA*) which is well reported to positively regulate the biofilm formation. SarA protein has high binding affinity to the promoter region of *ica* operon and induces the production of poly-N-acetylglucosamine (PNAG) also known as PIA which facilitates biofilm formation. Additionally, SarA induces the expression of biofilm-associated adhesive proteins. Further, the stress responsive sigma factor (σ^B) activates *sarA* as well as *ica* operon mediated PIA biosynthesis and supports biofilm formation. On the other hand, MgrA, a well-known member of *sarA* family, impedes biofilm formation by inhibiting the process of autolysis, and it is also involved in activation of *agr* system [57, 60].

18.5 Unveiling the Drug Targets in *S. aureus* Using Proteomic Approaches

18.5.1 Importance of Proteomics in Drug Target Discovery

In recent years, research on proteomics gained more attention because of its ability to extract, separate, analyze, and identify the total proteome. As proteins are functional players arising from genes and being involved in various cellular processes, research on proteome level will enlighten the actual molecular mechanisms of biologically active compounds. In addition, proteomic techniques are highly sensitive and reproducible against a wide range of proteins [61]. The discovery of drug target in any clinically important pathogen is important with a potential health benefits for the welfare of the society. Decoding the principal mechanism of action of a drug and analysis of off-target interactions are essential to explore the therapeutic potential and side effects of the drugs [62]. Proteomics is a robust approach to unveil the mode of action of biologically active molecules against the virulence traits of the pathogens. In addition, proteomics can be exploited to study the quantification of protein abundance, interaction of proteins with other biomacromolecules, and post-translational modifications [63]. The study of proteomics is commonly categorized into two, namely, bottom-up and top-down approaches. The top-down proteomic approach is used to analyze the complex proteins in the intact native state, whereas in the bottom-up approach, proteins are fragmented to peptides prior to analysis and identification. The bottom-up strategy is widely used in the field of health and medicine due to its sensitivity and reliability [64].

18.5.2 Entire Proteome of *S. aureus*

As genomics serves as the backbone of proteomics, publication of complete genome sequence of *S. aureus* in the year 2001 laid the foundation for *S. aureus* proteomics [65]. From the genome sequence, the number of open reading frames was predicted to be around 2600. Comprehensive mass spectrometric studies coupled with two-dimensional polyacrylamide gel electrophoresis (2-DGE) identified 1123 cytoplasmic proteins which represent 66% of predicated cytoplasmic proteins, and 2-DGE reference map (with 473 identified proteins) of *S. aureus* cellular proteome was first established in 2005 [66]. Later, the total proteome of *S. aureus* comprising cytoplasmic, surface-associated, membrane, and secreted proteome was predicted to be 2618 proteins of which 2005 proteins (77%) have been identified (Fig. 18.2) [67].

18.5.3 Proteomic Strategies of *S. aureus*

Proteomic strategies are basically composed of protein extraction, purification, separation, and identification. Gel-based separations of proteins are common and widely used in the field of comparative proteomics. Advancements in the mass

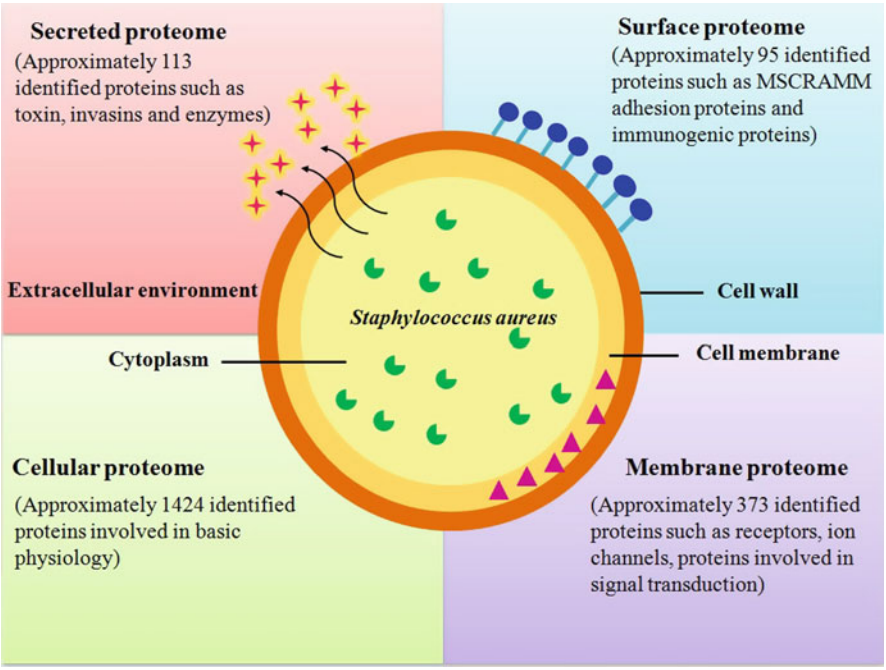

Fig. 18.2 Compendious proteome of *S. aureus* (Data obtained from [67])

spectrometric technologies enabled the gel-free quantification of proteins based on spectral counting and peak intensities [68]. Comprehensive workflow of *S. aureus* proteomic strategies is presented in Fig. 18.3.

One-dimensional SDS-polyacrylamide gel electrophoresis (SDS-PAGE) is the simplest gel-based proteomic technique used for the separation of proteins according to molecular weight. In case of crude protein samples, this technique is used for the purification of proteins prior to further analysis. Native PAGE analysis is generally used to identify the known protein targets in native form [69]. In 1975, 2-DGE was first introduced by O'Farrell and Klose and remains a gold standard proteomic technique for the separation of complex protein mixtures till date [70]. The workflow of 2-DGE comprises extraction and purification of proteins, rehydration, first dimensional separation based on isoelectric point otherwise known as isoelectric focusing, reduction, alkylation, second dimensional separation based on molecular weight, staining and visualization of protein spots, image analysis and in-gel digestion of proteins, mass spectrometry, and database search based identification [71]. Advancement of 2-DGE with the use of mass spectrometry compatible CyDyes led to an effective proteomic approach difference gel electrophoresis (DIGE). This technique excludes the gel-to-gel variations which are main disadvantage of 2-DGE and also provides extensive relative quantification of proteins [72]. Comparative gel-based analysis of protein samples from control and treated cells can identify the

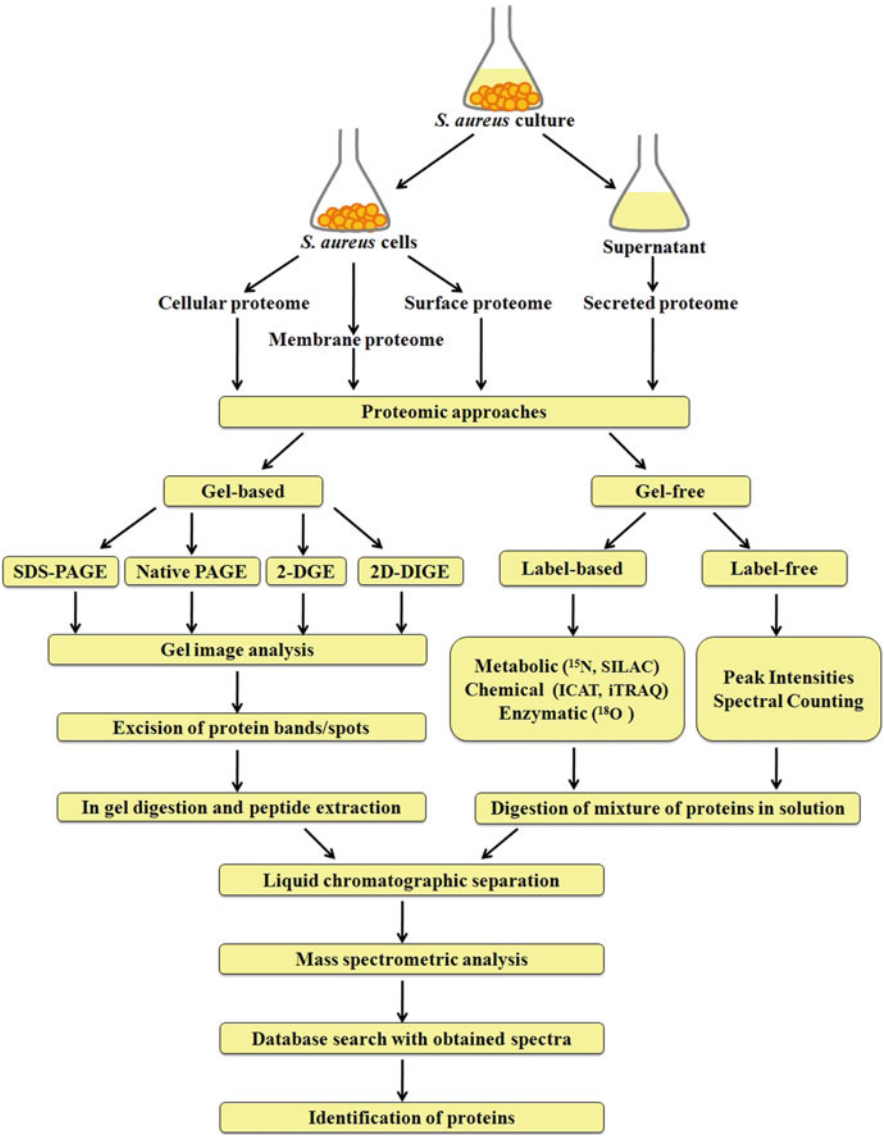

Fig. 18.3 Schematic illustration of comprehensive workflow involved in various strategies of *S. aureus* proteomics

differentially regulated proteins, and mass spectrometric identification of proteins spots can reveal the molecular protein targets of drugs [73].

2-DGE-based proteomic study revealed that rhodomyrtone interrupted cell wall biosynthesis and cell division in *S. aureus* to exert antibacterial activity [74]. Our previous study identified the multiple protein targets of citral to inhibit biofilm and

virulence. Citral upregulated the transcriptional repressor CodY which suppresses the major adhesion and secreted virulence factors [75]. 2-DGE-based proteomic analysis of cellular proteins of *S. aureus* treated with juglone exhibited the inhibition of DNA and RNA synthesis [76]. Quantitative proteomic analysis using isobaric tags unveiled inhibition of protein synthesis by 3-O-alpha-L-(2″,3″-di-p-coumaroyl) rhamnoside in *S. aureus* [77]. Spectral counting-based label-free quantitative proteomics of oxacillin-treated *S. aureus* revealed the upregulation of tolerance and resistance mechanisms [78]. Disruption of oxidation-reduction homeostasis and cell wall biosynthesis by combination of erythromycin and oxacillin was elucidated by spectral counting-based label-free proteomic approach [79]. Disruption of iron homeostasis induces SOS response in *S. aureus* upon treatment with punicalagin identified from pomegranate through quantitative isobaric labeling-based proteomic approach [80].

18.6 Concluding Remarks

This chapter demonstrated the pathogenesis, major virulence determinants, and biofilm formation of *S. aureus* and its clinical relevance. Alternative therapeutic developments of drug discovery to overcome the burden of antibiotic resistance are provided in detail. The importance of proteomics in the field of drug discovery and target identification and various proteomic strategies including gel-based and gel-free techniques in aspect of decoding the molecular targets of drugs are discussed. Understanding of *S. aureus* pathogenesis and current approaches for drug target identification will serve as platform for future studies for the development of effective strategies to combat *S. aureus* infections.

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Competing Interest
All the authors declare no conflict of interest.

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